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(71) Applicants *(for all designated States except US)*: **LUDWIG INSTITUTE FOR CANCER RESEARCH** [US/US]; 605 Third Avenue, New York, NY 10158 (US). **LICENTIA, LTD.** [FI/FI]; Erottajankatu 19 B, 6th floor, FIN-00130 Helsinki (FI).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ALITALO, Kari** [FI/FI]; Nyyrikintie 4 A, 02100 Espoo (FI). **HE, Yulong** [CN/FI]; Molecular/Cancer Biology Laboratory,, Biomedicum Helsinki, University of Helsinki, P.O. Box 63 (Haartmaninkatu 8), FIN-00014 Helsinki (FI). **TAMMELA, Toumas** [FI/FI]; Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, University of Helsinki, P.O. Box 63 (Haartmaninkatu 8), Fin-00014 Helsinki (FI).

(74) Agent: **GASS, Daciv, A.**; Marshall, Gerstein & Borun, LLP, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).

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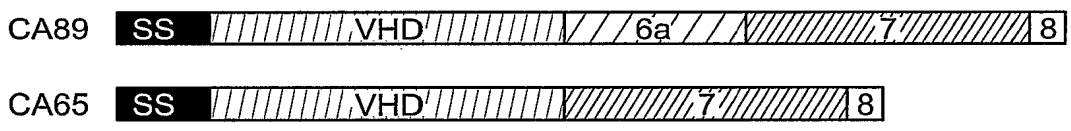
(54) Title: HEPARIN BINDING VEGER-3 LIGANDS



(57) Abstract: The present invention is directed to methods and compositions for making and using chimeric polypeptides that comprise a VEGFR-3 ligand and a heparin binding domain. The chimeric molecules of the present invention retain VEGFR-3 binding activity and an enhanced heparin binding activity as compared to native VEGF-C and/or VEFG-D.

WO 2005/016963 A2

HEPARIN BINDING VEGFR-3 LIGANDS

Cross-Reference to Prior Applications

The present application is a continuation-in-part of U.S. Patent Application No. 10/669,176 filed September 23, 2003. The present application claims the benefit of priority of U.S. Provisional Patent Application No. 60/478,390, which was filed on filed on June 12, 2003. The present application also claims the benefit of priority of U.S. Patent No. 60/478,114, filed June 12, 2003. The entire text of each of the foregoing applications is specifically incorporated herein by reference.

Field of the Invention

The present application is directed to methods and compositions for promoting lymphangiogenesis and/or angiogenesis. The application describes chimeric polypeptides that comprise a VEGFR-3 ligand and a heparin binding domain and methods and compositions for using the same.

Background

The VEGF proteins and their receptors (VEGFRs) play important roles in both vasculogenesis, the development of the embryonic vasculature from early differentiating endothelial cells, and angiogenesis, the process of forming new blood vessels from pre-existing ones (Risau *et al., Dev Biol 125*:441-450 (1988); Zachary, *Intl J Biochem Cell Bio 30*:1169-1174, 1998; Neufeld *et al., FASEB J 13*:9-22, 1999); Ferrara, *J Mol Med 77*:527-543, 1999). Both processes depend on tight control of endothelial cell proliferation, migration, differentiation, and survival. Dysfunction of the endothelial cell regulatory system is a key feature of cancer and several diseases associated with abnormal angiogenesis, such as proliferative retinopathies, age-related muscular degeneration, rheumatoid arthritis, and psoriasis. Understanding of the specific biological function of the key players involved in regulating endothelial cells will lead to more effective therapeutic applications to treat such diseases (Zachary, *Intl J Biochem Cell Bio 30*:1169-1174, 1998; Neufeld *et al., FASEB J 13*:9-22, 1999; Ferrara, *J Mol Med 77*:527-543, 1999).

The mammalian vascular endothelial growth factor (VEGF) family members identified to date, including VEGF, VEGF-B, VEGF-C, VEGF-D, and

placenta growth factor (PlGF), play crucial roles in the physiological and pathological regulation of vasculogenesis, hematopoiesis, angiogenesis, lymphangiogenesis, and vascular permeability (Ferrara and Davis-Smyth, *Endocr Rev* 18: 4-25, 1997; Veikkola *et al., Cancer Res* 60: 203-12, 2000; Carmeliet and Jain, *Nature*, 407:249-57, 2000). VEGF, also identified as a potent vascular permeability-enhancing factor (Dvorak, *et al., Am J Pathol* 146: 1029-39, 1995), is a potent growth factor for blood vessel formation and plays an essential role in this process (Ferrara and Davis-Smyth, *Endocr Rev* 18: 4-25, 1997].

It has been noted that both insufficient and excessive VEGF lead to abnormal blood vessel formation. This property and the permeability-inducing property of VEGF may pose difficulties for its *in vivo* application. Both VEGF-C and VEGF-D have been shown to induce lymphangiogenesis in transgenic mice and in other *in vivo* models (Jeltsch *et al., Science* 276:1423-5, 1997; Oh *et al., Dev Biol* 188: 96-109, 1997; Veikkola *et al., EMBO J* 20: 1223-31, 2001). VEGF-C and VEGF-D signal primarily through VEGFR-3 [Veikkola *et al., EMBO J* 20: 1223-31, 2001; Joukov *et al., EMBO J* 15: 290-98 1996; Lee *et al., Proc Natl Acad Sci U S A* 93: 1988-92, 1996; Achen *et al., Proc Natl Acad Sci U S A* 95: 548-53, 1998; Makinen *et al., Nat Med* 7: 199-205, 2001].

VEGF-C and VEGF-D are produced as precursor proteins with N- and C-terminal pro-peptides flanking the VEGF homology domain (VHD; Joukov *et al., EMBO J.* 16:3898-3011, 1997). A schematic view of the VEGF-C prepro-peptide is shown in Fig. 1A. The proteolytic processing of prepro-polypeptides of VEGF-C and VEGF-D increases their affinities for VEGFR-3, and the fully-processed mature forms can also bind to, and activate, VEGFR-2 (Joukov *et al., EMBO J* 16: 3898-911, 1997; Stacker *et al., J Biol Chem* 274 32127-36, 1999). Both factors can thus theoretically exert angiogenic activity via VEGFR-2.

However, in transgenic models in which both wild type and mutant forms of VEGF-C induced lymphangiogenesis in the skin, no angiogenic effect was observed. It was therefore suggested that during embryonic development VEGF-C may not be fully processed to a form that activates the VEGFR-2 of blood vessels [Jeltsch *et al., Science* 276:1423-5, 1997; Veikkola et al., *Embo J* 20: 1223-31, 2001]. In addition, although the recombinant mature form of VEGF-C has been shown to induce angiogenesis and lymphangiogenesis (Cao *et al. Proc Natl Acad Sci U S A* 95:

14389-94, 1998; Marconcini *et al., Proc Natl Acad Sci U S A* 96: 9671-6, 1999), its angiogenic activity was weak when it was delivered through viral vectors such as adenoviral or adeno-associated viral vector.

Thus, while VEGF-C and VEGF-D have been shown to have significant angiogenic and lymphangiogenic effects in a number of settings, there remains a desire to enhance the angiogenic and/or lymphangiogenic effects of these molecules to render them more efficacious in these indications.

Summary of the Invention

The present invention addresses the need for more efficacious angiogenic and lymphangiogenic VEGF molecules by providing chimeric polypeptides that comprise a VEGF homology domain (VHD) and a heparin binding domain.

The invention includes numerous aspects and embodiments described throughout the application. In certain exemplary embodiments, the present invention is a compound comprising the formula **X-B-Z** or **Z-B-X**, wherein **X** binds Vascular Endothelial Growth Factor Receptor 3 (VEGFR-3) and comprises an amino acid sequence at least 70%, or more preferably 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a VEGFR-3 ligand selected from the group consisting of:

(a) the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2;

(b) fragments of (a) that bind VEGFR-3;

(c) the prepro-VEGF-D amino acid sequence set forth in SEQ ID NO: 4; and

(d) fragments of (c) that bind VEGFR-3;

wherein **Z** comprises a heparin-binding amino acid sequence; and

wherein **B** comprises a covalent attachment linking **X** to **Z**.

In particular embodiments, it is contemplated that the compound comprises the formula **X-B-Z** or **Z-B-X**, wherein **X** binds Vascular Endothelial Growth Factor Receptor 3 (VEGFR-3) and comprises an amino acid sequence at least 70%, or more preferably 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a VEGFR-3 ligand that is a chimera comprised of sequences of two or more vacular endothelial growth factors, including the chimeras described in WO 01/62942; wherein **Z** comprises a heparin-binding amino acid sequence, with the proviso that the heparin binding sequence is not identical to a VEGF-A heparin binding sequence; and wherein **B** comprises a covalent attachment linking **X** to **Z**.

The compounds described above may also preferably bind Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2). The compounds of the invention advantageously comprise a heparin binding domain that facilitates the biological bioavailability of the growth factor. As such, in the chimeric compounds of the invention the moiety **Z** may be any heparin binding domain that retains an heparin binding activity when joined to a growth factor as described herein. In specific embodiments, the heparin binding amino acid sequence is derived from a vascular endothelial growth factor. In exemplary embodiments, the sequence comprises an amino acid sequence at least 70% identical, or more preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical, to a sequence selected from the group consisting of:

(a) amino acids 142-165 of the $VEGF_{206}$ (amino acids encoded by exon 6a of VEGF);

(b) amino acids 183 to 226 of the $VEGF_{206}$ (amino acids encoded by exon 7 of VEGF);

(c) amino acids 142-165 joined directly to amino acids 183-226 of the $VEGF_{206}$ (amino acids encoded by exons 6 through 7 of VEGF);

(d) amino acids 142 to 226 of the $VEGF_{206}$ (amino acids encoded by exons 6 though 8 of VEGF);

(e) amino acids 138 to 182 of the VEGF-B_{167} sequence set forth in SEQ ID NO: 8;

(f) amino acids 193 to 213 of the PlGF-3 sequence set forth in SEQ ID NO: 15;

(g) amino acids of 142 to 162 of the PlGF-2 sequence set forth in SEQ ID NO: 69;

(h) fragments of (a) - (g) that bind heparin.

Additional exemplary embodiments comprise a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the heparin binding regions of many additional sequences identified in the detailed description. Human sequences are preferred for molecules to be administered to humans.

In particularly preferred embodiments, the moiety defined by the formula **X-B-Z** or **Z-B-X**, defines a polypeptide. Preferably, such a polypeptide further comprises a signal peptide at the amino terminus of the polypeptide, wherein the signal peptide directs secretion of a polypeptide comprising **X-B-Z** or **Z-B-X** from a cell that expresses the polypeptide.

In the compositions described herein, the moiety **B** is a linking moiety that links X and Z. Such a moiety may be a peptide bond or alternatively may be a peptide or other organic linker commonly used in the preparation of chimeric molecules. The peptide linker may, for example be an amino acid linker up to 500 amino acids. In other specific embodiments, **B** comprises a peptide bond that is cleavable by an agent that fails to cleave the amino acid sequence **X** that binds VEGFR-3. More particularly, such a peptide bond is preferably cleaved by a protease. In exemplary specific embodiments, the moiety **B** comprises an amino acid sequence that contains a protease cleavage site selected from the group consisting of a Factor Xa cleavage site, an enterokinase cleavage site, a thrombin cleavage site, a TEV cleavage site, and a PreScission cleavage site. In other preferred embodiments, the moiety **B** comprises an amino acid sequence of at least four, and more preferably at least six amino acids from a VEGF-C or VEGF-D amino acid sequence, wherein the at least four amino acids are cleaved *in vivo* to separate an amino-terminal VEGF-C or VEGF-D propeptide from a mature VEGF-C or VEGF-D protein.

It is particularly contemplated that in the compounds of the invention **X** comprises an amino acid sequence at least 95% identical to the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2 or to a fragment thereof that binds VEGFR-3. Other embodiments contemplate that **X** comprises an amino acid sequence at least 95% identical to the prepro-VEGF-C amino acid sequence set forth

in SEQ ID NO: 2 or to a fragment thereof that binds VEGFR-3, with the proviso that the cysteine corresponding to amino acid position 156 of SEQ ID NO: 2 has been deleted or replaced with an amino acid other than cysteine, and the resultant amino acid sequence binds VEGFR-3 but has reduced VEGFR-2 binding. Such VEGF-C ΔC_{156} polypeptides are described in detail in International Patent Publication No. WO 98/33917, incorporated herein by reference.

In still other examples of the compounds useful in the present invention, **X** comprises an amino acid sequence identical to the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2 or to a fragment thereof that binds VEGFR-3. In yet further embodiments, **X** may comprise an amino acid sequence identical to the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2 or to a fragment thereof that binds VEGFR-3, with the proviso that the cysteine corresponding to amino acid position 156 of SEQ ID NO: 2 has been deleted or replaced with an amino acid other than cysteine, and the resultant amino acid sequence binds VEGFR-3 but has reduced VEGFR-2 binding.

In alternative embodiments, **X** may comprise an amino acid sequence at least 95% identical to the prepro-VEGF-D amino acid sequence set forth in SEQ ID NO: 4 or to a fragment thereof that binds VEGFR-3. Other specific embodiments contemplate that **X** comprises an amino acid sequence identical to the prepro-VEGF-D amino acid sequence set forth in SEQ ID NO: 4 or to a fragment thereof that binds VEGFR-3.

It is particularly contemplated that any of the compounds of the invention may be prepared to further include a peptide tag, e.g., a polyhistidine tag. Inclusion of such a tag may facilitate purification. In additional embodiments, the compounds may be PEGylated with one or more polyethylene glycol (PEG) moieties.

The compounds of the present invention may advantageously be formulated into compositions wherein such compositions comprise a compound of the invention in a pharmaceutically acceptable carrier. The compounds of the invention are preferably useful in the manufacture of medicaments. For example, the compounds of the invention have a use in the manufacture of a medicament for modulation of VEGFR-3 and/or VEGFR-2 to treat diseases or conditions that would benefit from such modulation.

Other compositions of the present invention describe polynucleotides that comprising a nucleotide sequence that encodes a chimeric protein compound of formula **X-B-Z** or **Z-B-X** as discussed above and described in further detail in the description below. In specific embodiments, the polynucleotide further comprises a nucleotide sequence that encodes a signal peptide fused in-frame with the polypeptides described above. Vectors that comprise such polynucleotides also are contemplated. The present invention particularly contemplates an expression vector comprising a polynucleotide comprising a nucleotide sequence that encodes a chimeric protein compound of formula **X-B-Z** or **Z-B-X** operably linked to an expression control sequence. In certain embodiments, the expression control sequence is an endothelial cell specific promoter. The expression vector may be any vector used for the expression of a nucleic acid and may for example, be selected from the group consisting of replication deficient adenoviral vectors, adeno-associated viral vectors, and lentivirus vectors. The polynucleotides and vectors of the invention may be formulated as a compositions in which the polynucleotide or the vector is presented in a pharmaceutically acceptable carrier. The polynucleotides or vectors according to the invention may be used in the manufacture of a medicament for modulation of VEGFR-3 and/or VEGFR-2, to treat diseases or conditions that would benefit from such modulation.

Also contemplated are host cells that have been transformed or transfected with a polynucleotide or vector of the invention.

Other aspects of the invention are directed to methods of modulating the growth of mammalian endothelial cells or mammalian endothelial precursor cells, comprising contacting the cells with a composition comprising a member selected from the group consisting of a polypeptide compound of formula **X-B-Z** or **Z-B-X**; a polynucleotide that encodes such a compound; an expression vector containing such a polynucleotide operatively linked to an expression control sequence; and a cell transformed or transfected with such a polynucleotide or such a vector that expresses the polypeptide compound of formula **X-B-Z** or **Z-B-X**. In certain embodiments, the contacting comprises administering the composition to a mammalian subject in an amount effective to modulate endothelial cell growth *in vivo*. In particular embodiments, the mammalian subject is a human.

Also contemplated herein is a method of modulating growth of mammalian hematopoietic progenitor cells, comprising contacting the cells with a composition comprising a member selected from the group consisting of polypeptide compound of formula **X-B-Z** or **Z-B-X**; a polynucleotide that encodes such a compound; an expression vector containing such a polynucleotide operatively linked to an expression control sequence; and a cell transformed or transfected with such a polynucleotide or such a vector that expresses the polypeptide compound of formula **X-B-Z** or **Z-B-X**.

The methods described herein may be used for the activation of VEGFR-3. Such methods would generally comprise contacting cells that express VEGFR-3 with a composition comprising a polypeptide compound of formula **X-B-Z** or **Z-B-X**.

Other embodiments of the invention are directed to methods of stimulating lymphangiogenesis in a mammal comprising contacting said mammal with, and/or administering to said mammal, a composition comprising a member selected from the group consisting of polypeptide compound of formula **X-B-Z** or **Z-B-X**; a polynucleotide that encodes such a compound; an expression vector containing such a polynucleotide operatively linked to an expression control sequence; and a cell transformed or transfected with such a polynucleotide or such a vector that expresses the polypeptide compound of formula **X-B-Z** or **Z-B-X**.

Also contemplated are methods of stimulating angiogenesis in a mammal comprising contacting said mammal with a composition comprising a member selected from the group consisting of

(a) a polypeptide compound of formula **X-B-Z** or **Z-B-X** wherein the compound binds Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2); a polynucleotide that encodes such a polypeptide compound, an expression vector containing such a polynucleotide operatively linked to an expression control sequence; and a cell transformed or transfected with such a polynucleotide or vectors that expresses the polypeptide compound of formula **X-B-Z** or **Z-B-X** wherein the compound binds Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2).

It has been shown that VEGF-C functions as a neurotrophic and neuroprotective growth factor (for detailed description see U.S. Patent Application

No. 10/669,176, filed September 23, 2003, incorporated herein by reference in its entirety). As such, the compositions of the present invention may be used alone or in combination with additional agents to treat disorders in which neuronal loss or functional deficiency is a problem.

In specific embodiments, the disease or disorder being treated is a neurodegenerative disorder, wherein the neurodegenerative disorder is selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease, Amyotrophic Lateral Sclerosis (ALS), dementia and cerebral palsy. In another embodiment, the disease or condition is selected from the group consisting of neural trauma or neural injury. Methods of the invention also can be performed to treat or ameliorate the effects of neural trauma or injury, such as injury related to stroke, spinal cord injury, post-operative injury, brain ischemia and other traumas. Patients affected by any of the above disorders are administered a polypeptide of formula **X-B-Z** or **Z-B-X**, either systemically, or preferably at the site of neuropathology, to stimulate the proliferation of neural stem cells *in vivo*. As described above for other indications, administration of polynucleotides, expression vectors, and transformed cells is specifically contemplated for neurological indications. Alternatively, patients are administered neural stem cells isolated from a biological sample, from a commercial source or an immortalized neural stem cell, which have been transformed to express a polypeptide of formula **X-B-Z** or **Z-B-X**. The neural stem cells are then administered to a patient with a neurodegenerative disorder or neural trauma such that they will migrate to the site of neural degeneration and proliferate. In a related variation, neuronal stem cells are cultured *ex vivo* with polypeptides of the invention before administration.

Thus, an aspect of the invention is a method of promoting recruitment, proliferation, differentiation, migration or survival of neuronal cells or neuronal precursor cells in a mammalian subject comprising administering to the subject a composition comprising a vascular endothelial growth factor C (VEGF-C) product or a vascular endothelial growth factor D (VEGF-D) product, wherein the VEGF-C or VEGF-D product is a heparin binding polypeptide as described herein, or a polynucleotide that encodes such a polypeptide, or vector comprising such a polynucleotide, or a host cell that expresses such a polypeptide. In preferred variations, the method further comprises a step, prior to the administrating step, of

identifying a mammalian subject in need of neuronal cell or neuronal precursor cell recruitment, proliferation, or differentiation. Candidates include subjects having disorders described in the preceding paragraph.

Combination therapy is specifically contemplated for neurological therapies, such as co-administration of the VEGF-C or VEGF-D product in conjunction with a neural growth factor. Exemplary factors include interferon gamma, nerve growth factor, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), neurogenin, brain derived neurotrophic factor (BDNF), thyroid hormone, bone morphogenic proteins (BMPs), leukemia inhibitory factor (LIF), sonic hedgehog, glial cell line-derived neurotrophic factor (GDNFs), vascular endothelial growth factor (VEGF), interleukins, interferons, stem cell factor (SCF), activins, inhibins, chemokines, retinoic acid and ciliary neurotrophic factor (CNTF).

Compositions comprising polypeptides of the invention and any of the foregoing polypeptides, or comprising one or more polynucleotides that encode polypeptides of the invention and any of the foregoing polypeptides, are specifically contemplated as an aspect of the invention.

In yet another aspect, the invention provides compositions and methods of treatment involving polypeptides of the invention (e.g., polypeptides of formula X-B-Z or Z-B-X, or polynucleotides encoding them) in combination with other polypeptides that will enhance vessel formation or integrity, are specifically contemplated. The polypeptides (or encoding polynucleotides) specifically contemplated include, but are not limited to, Angiopoietin-1 (Ang-1, SEQ ID NO: 67), PDGF-A, PDGF-B, PDGF-C, PDGF-D, VEGF, VEGF-B, and combinations thereof. Such combinations will be useful in the optimal induction of functional vessels, such as lymphatic vessels.

The foregoing summary is not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document. Where protein therapy is described, embodiments involving polynucleotide therapy (using polynucleotides that

encode the protein) are specifically contemplated, and the reverse also is true. Where embodiments of the invention are described with respect to VEGF-C, it should be appreciated that analogous embodiments involving VEGF-D are specifically contemplated.

In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations defined by specific paragraphs above. For example, certain aspects of the invention that are described as a genus, and it should be understood that every member of a genus is, individually, an aspect of the invention. Also, aspects described as a genus or selecting a member of a genus, should be understood to embrace combinations of two or more members of the genus. Although the applicant(s) invented the full scope of the invention described herein, the applicants do not intend to claim subject matter described in the prior art work of others. Therefore, in the event that statutory prior art within the scope of a claim is brought to the attention of the applicants by a Patent Office or other entity or individual, the applicant(s) reserve the right to exercise amendment rights under applicable patent laws to redefine the subject matter of such a claim to specifically exclude such statutory prior art or obvious variations of statutory prior art from the scope of such a claim. Variations of the invention defined by such amended claims also are intended as aspects of the invention.

Brief Description of the Drawing

The following drawings form part of the present specification and are included to further illustrate aspects of the present invention. The invention may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

Fig. 1A schematically depicts the proteolytic processing of VEGF-C (Joukov *et al.*, *EMBO J* 16: 3898-911, 1997). SS, signal sequence; N-term and C-term, N-terminal and C-terminal (silk homology domain) propeptides; VHD, VEGF homology domain; arrowheads, cleavage sites; and disulfide bonds are marked as –S-S- and dotted lines as non-covalent bonds.

Fig. 1B schematically depicts VEGF splice variants (named $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$) generated by alternative splicing of the eight exons (numbered 1 to 8 shown at the bottom) of the human VEGF gene.

Fig. 1C is a schematic illustration of two VEGF-C/VEGF chimeric molecules comprised of the signal sequence and the VEGF homology domain of VEGF-C, and VEGF exon 6-8 or exon 7-8 encoded sequences (CA89 and CA65, respectively).

Fig. 1D is an autoradiogram depicting immunoprecipitation analysis of radiolabeled, secreted proteins in the conditioned medium from the 293T cells transfected with pEBS7/CA89 (with or without heparin 20 unit/ml incuded in the medium), pEBS7/CA65 or the pEBS7 vector alone.

Fig. 2 is a graph depicting absorbance measurements (540 nm wavelength) of reaction products in a cell viability assay to measure biological activity of the chimeric molecules depicted in Figure 1C-1D. The biological activity of the VEGF-C chimeric proteins was demonstrated by a bioassay using Ba/F3 cells expressing a chimeric VEGFR-3/erythropoietin (Epo) receptor which transmitted survival and proliferation signals of VEGF-C for the IL-3 dependent Ba/F3/VEGFR-3 cells. Data represent the mean values from triplicate assays.

Fig. 3A. Immunoprecipitation and polyacrylamide gel electrophoresis of secreted proteins (labeled with ^{35}S) from the conditioned medium of 293T cells transfected with pEBS7/CA89 (CA89), pEBS7/CA65 (CA65), pEBS7/VEGF-C N C (N C), or the pEBS7 vector, with neuropilin-1-Ig (NP1) and neuropilin-2-Ig (NP2)

Fig. 3B. Immunoprecipitation and polyacrylamide gel electrophoresis of secreted proteins (labeled with ^{35}S) from the conditioned medium of 293T cells transfected with pEBS7/CA89 (CA89), pEBS7/CA65 (CA65), pEBS7/VEGF-CΔNΔC (NΔC), or the pEBS7 vector, with VEGFR-1-Ig (R-1), VEGFR-2-Ig (R-2) and VEGFR-3-Ig (R-3).

Fig. 4A. Analysis of viral expression of the chimeric molecules. Recombinant AAV (A) expression of CA89, CA65, VEGF-CΔNΔC and VEGF-C were analysed by immunoprecipitation of metabolically labelled proteins with anti-VEGF-C serum followed by SDS-PAGE under reducing conditions.

Fig. 4B. Analysis of viral expression of the chimeric molecules. Recombinant adenoviral expression of CA89, CA65, VEGF-CΔNΔC and VEGF-C were analysed by immunoprecipitation of metabolically labelled proteins with anti-VEGF-C serum followed by SDS-PAGE under reducing conditions.

5

Detailed Description of the Preferred Embodiments

VEGF-C and VEGF-D are ligands for Flt4 receptor tyrosine kinase, also known as Vascular Endothelial Growth Factor Receptor 3 (VEGFR-3). VEGFR-3 is primarily present on lymphatic endothelia and through interaction with this
10 receptor, these factors are thought to mediate lymphangiogenesis. Angiogenic effects of VEGF-C and VEGF-D are thought to be mediated through VEGFR-2. However, mature forms of VEGF-C delivered by means such as adenoviral gene therapy vectors induced only weak lymphangiongiogenic activity and little angiogenic activity, if any, in mice.

15 This weak activity suggests that the concentration of the protein present may not be sufficient, or that the half-life of the mature form of VEGF-C protein may be too short, to induce a potent angiogenic effect. VEGF, which has potent angiogenic activity, includes a heparin binding domain. $VEGF_{121}$ has potent angiogenic activity, but does not contain a heparin binding domain. The major forms
20 of VEGF are $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$, which result from alternative RNA splicing (Fig. 1 B) (Ferrara and Davis-Smyth, *Endocr Rev* 18: 4-25, 1997). An important biological property that distinguishes these VEGF isoforms from each other is their different binding affinities to heparin and heparan sulfate. The four longer isoforms described above contain a heparin binding domain encoded
25 by exon 6 and/or exon 7. The 21 amino acids encoded by exon 6 contain a heparin binding domain and also elements that enable binding to extracellular matrix (Poltorak *et al., J. Biol. Chem.* 272:7151-8, 1997). Molecules containing the cationic polypeptide sequence encoded by exon 7 (44 amino acids) are also heparin-binding and remain bound to the cell surface and the extracellular matrix. Recently, it has
30 been shown that carboxymethyl benzylamide dextran, a heparin-like molecule, effectively inhibits the activity of $VEGF_{165}$ by interfering with heparin binding to $VEGF_{165}$ (Hamma-Kourbali *et al., J Biol Chem.*, 276(43):39748-54, 2001). There is also other evidence that points to the importance of the heparin binding property of

growth factors for their biological activities (Dougher *et al., Growth Factors,* 14: 257-68, 1997; Carmeliet *et al., Nat Med* 5: 495-502, 1999; Ruhrberg *et al., Genes Dev* 16 2684-98, 2002).

VEGF-C and VEGF-D do not have significant heparin binding activity (and, for the purposes of this invention, are not "heparin binding" as that term is used). In order to achieve maximum activation of VEGFR-2 and VEGFR-3 *in vivo,* and produce VEGF-C and/or VEGF-D molecules that are more potent in inducing angiogenesis and/or lymphangiogenesis, the inventors have produced or described chimeric molecules of VEGF-C and VEGF-D in which the VHD domain is fused or otherwise linked to a heparin binding domain. Methods and compositions for making and using these molecules are described in further detail herein below.

A. Chimeric Molecules of the Present Invention

The present invention provides chimeric VEGFR-3 ligands of the formula **X-B-Z** or **Z-B-X**, where domain **X** binds Vascular Endothelial Growth Factor Receptor 3 (VEGFR-3) and domain **Z** comprises a heparin binding amino acid sequence. "Domain" **B,** which comprises a covalent attachment linking **X** to **Z**, and at its simplest, is nothing more than a peptide bond or other covalent bond Preferably, domain **X** comprises an amino acid sequence at least 90% identical to a prepro-VEGF-C amino acid sequence, a fragment of VEGF-C that possesses VEGFR3 binding activity, a prepro-VEGF-D amino acid sequence, or a fragment of VEGF-D that possesses VEGFR3 binding activity. These and other molecules that may serve as **X** are described in further detail herein.

The chimeric molecules of the present invention are engineered to possess a heparin binding domain **Z** which preferably increases potency of the molecule as an inducer of angiogenesis and/or lymphangiogenesis, as compared to a similar VEGFR-3 ligand that lacks a heparin binding domain (such as wildtype VEGF-C or -D). This increase in potency may, for example, be due to an increase in the half-life of the chimeric molecule *in vivo* as compared to the unmodified VEGFR-3 ligand, or to better or more sustained localization in the bloodstream, lymph, or vessel tissues, or other tisses.

a. Domain X: a VEGFR-3 binding domain

The VEGFR-3 ligand binding domain of molecules of the invention can be any amino acid sequence that binds VEGFR-3, and confers VEGFR-3 binding to the molecules of the invention. For the purposes of the invention, VEGFR-3 binding means binding to the extracellular domain of human VEGFR-3 (Flt4 receptor tyrosine kinase) as described in U.S. Patent No. 5,776,755, incorporated herein by reference. Molecules that have at least 10% of the binding affinity of fully-processed (mature) human VEGF-C or VEGF-D for VEGFR-3 are considered molecules that bind VEGFR-3.

Preferred VEGFR-3 binding domains share significant amino acid similarity to a naturally occurring vertebrate VEGF-C or VEGF-D, many of which have been described in the literature and others of which can be cloned from genomic DNA or cDNA libraries, and using PCR and/or standard hybridization techniques and using known VEGF-C or -D cDNAs as probes. For example, preferred molecules have at least 70% amino acid identity to a naturally occurring VEGF-C or -D protein or to a fragment thereof that binds VEGFR-3. Still more preferred are VEGFR-3 binding domains with at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity with the natural/wild type vertebrate VEGFR-3 ligand sequence. Descriptions herein of embodiments involving wild type sequences should be understood also to apply to variants sharing such amino acid similarity. It will be appreciated that conservative substitutions and/or substitutions based on sequence alignments with species homologues are less likely to diminish VEGFR-3 binding activity compared to the wild type reference sequence.

A very highly preferred wild type VEGFR-3 ligand for use as the VEGFR-3 binding domain is human prepro-VEGF-C and VEGFR-3 binding fragments thereof. Human VEGF-C polypeptides that may be used as domain **X** are described in WO 97/05250, WO 98/33917, WO 00/24412, and U.S. Patent Nos. 6,221,839, 6,361,946, 6,645,933, 6,730,658 and 6,245,530, each of which is incorporated herein by reference in its entirety.

VEGF-C comprises a VHD that is approximately 30% identical at the amino acid level to VEGF. VEGF-C is originally expressed as a larger precursor protein, prepro-VEGF-C, having extensive amino- and carboxy-terminal peptide

sequences flanking the VHD, with the C-terminal peptide containing tandemly repeated cysteine residues in a motif typical of Balbiani ring 3 protein. The nucleic acid and amino acid sequences of human prepro-VEGF-C are set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. Prepro-VEGF-C undergoes extensive proteolytic maturation involving the successive cleavage of a signal peptide, the C-terminal pro-peptide, and the N-terminal pro-peptide, as described in Joukov *et al.* (*EMBO J., 16:(13)*:3898-3911, 1997) and in the above-referenced patents. Secreted VEGF-C protein consists of a non-covalently linked homodimer, in which each monomer contains the VHD. The intermediate forms of VEGF-C produced by partial proteolytic processing show increasing affinity for the VEGFR-3 receptor, and the mature protein is also able to bind to the VEGFR-2 receptor. (Joukov *et al.*, *EMBO J., 16:(13)*:3898-3911, 1997). The entire text of U.S. Patent No. 6,361,946 is incorporated herein by reference as providing a teaching of the sequence of the VEGF-C protein, gene and mutants thereof.

For treatment of humans, VEGF-C polypeptides with an amino acid sequence of a human VEGF-C are highly preferred, and polynucleotides comprising a nucleotide sequence of a human VEGF-C cDNA are highly preferred. By "human VEGF-C" is meant a polypeptide corresponding to a naturally occurring protein (prepro-protein, partially-processed protein, or fully-processed mature protein) encoded by any allele of the human VEGF-C gene, or a polypeptide comprising a biologically active fragment of a naturally-occurring mature protein. By way of example, a human VEGF-C comprises a continuous portion of the amino acid sequence set forth in SEQ ID NO: 2 sufficient to permit the polypeptide to bind VEGFR-3 in cells that express VEGFR-3. A polypeptide comprising amino acids 131-211 of SEQ ID NO: 2 is specifically contemplated. For example, polypeptides having an amino acid sequence comprising a continuous portion of SEQ ID NO: 2, the continuous portion having, as its amino terminus, an amino acid selected from the group consisting of positions 30-131 of SEQ ID NO: 2, and having, as its carboxyl terminus, an amino acid selected from the group consisting of positions 211-419 of SEQ ID NO: 2 are contemplated. As explained elsewhere herein in greater detail, VEGF-C biological activities, especially those mediated through VEGFR-2, increase upon processing of both an amino-terminal and carboxyl-terminal pro-peptide. Thus, an amino terminus selected from the group consisting of positions 102-131 of SEQ ID

NO: 2 is preferred, and an amino terminus selected from the group consisting of positions 103-113 of SEQ ID NO: 2 is highly preferred. Likewise, a carboxyl terminus selected from the group consisting of positions 211-227 of SEQ ID NO: 2 is preferred. As stated above, the term "human VEGF-C" also is intended to encompass polypeptides encoded by allelic variants of the human VEGF-C characterized by the sequences set forth in SEQ ID NOs: 1 & 2.

Moreover, since the therapeutic VEGF-C is to be administered as recombinant VEGF-C or indirectly via somatic gene therapy, it is within the skill in the art (and an aspect of the invention) to make and use analogs of human VEGF-C (and polynucleotides that encode such analogs) wherein one or more amino acids have been added, deleted, or replaced with other amino acids, especially with conservative replacements, and wherein the VEGFR-3 binding activity has been retained. Analogs that retain VEGFR-3 binding biological activity are contemplated as VEGF-C polypeptides for use in the present invention. In a preferred embodiment, analogs having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 such modifications and that retain VEGFR-3 binding activity are contemplated as VEGF-C polypeptides for use in the present invention. Polynucleotides encoding such analogs are generated using conventional PCR, site-directed mutagenesis, and chemical synthesis techniques. Molecules that bind and stimulate phosphorylation of VEGFR-3 are preferred.

Conservative substitutions include the replacement of an amino acid by a residue having similar physicochemical properties, such as substituting one aliphatic residue (Ile, Val, Leu or Ala) for another, or substitution between basic residues Lys and Arg, acidic residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Further information regarding making phenotypically silent amino acid exchanges may be found in Bowie et al., Science 247:1306 1310 (1990).

In another variation, the VEGR-3 binding domain has an amino acid sequence similar to or identical to a mutant VEGF-C, in which a single cysteine (at position 156 of the human prepro-VEGF-C sequence) is either substituted by another amino acid or deleted (SEQ ID NO: 68). Such VEGF-CΔCys$_{156}$ (SEQ ID NO: 68) mutants, even when fully processed by removal of both pro-peptides, fail to bind VEGFR-2 but remain capable of binding and activating VEGFR-3. Such

polypeptides are described in International Patent Publication No. WO 98/33917 and U.S. Patent Nos. 6,130,071, and 6,361,946, each of which is incorporated herein by reference in its entirety, especially for their teachings of VEGF-C ΔCys_{156} molecules which may be used in producing chimeras of the present invention which comprise VEGF-C ΔCys_{156} as subunit X of the chimera.

Another highly preferred wild type VEGFR-3 ligand for use in constructing chimeric molecules of the invention is human VEGF-D. VEGF-D is initially expressed as a prepro-peptide that undergoes N-terminal and C-terminal proteolytic processing, and forms non-covalently linked dimers. VEGF-D stimulates mitogenic responses in endothelial cells *in vitro*. Exemplary human prepro-VEGF-D nucleic acid and amino acid sequences are set forth in SEQ ID NO:3 and SEQ ID NO:4, respectively. In addition, VEGF-D is described in greater detail in International Patent Publication No. WO 98/07832 and U.S. Patent No. 6,235,713, each of which is incorporated herein by reference and describes VEGF-D polypeptides and variants thereof that are useful in producing the chimeras of the present invention. VEGF-D related molecules also are described in International Patent Publication Nos. WO 98/02543 and WO 97/12972, and U.S. Patent No. 6,689,580, and U.S. Patent Application Nos. 09/219,345 and 09/847,524, all of which are incorporated by reference.

Isolation of a biologically active fragment of VEGF-D designated VEGF-DΔNΔC, is described in International Patent Publication No. WO 98/07832, incorporated herein by reference. VEGF-DΔNΔC consists of amino acid residues 93 to 201 of VEGF-D linked to the affinity tag peptide FLAG®. The prepro-VEGF-D polypeptide has a putative signal peptide of 21 amino acids and is apparently proteolytically processed in a manner analogous to the processing of prepro-VEGF-C. A "recombinantly matured" VEGF-D lacking residues 1-92 and 202-354 of SEQ ID NO: 4 retains the ability to activate receptors VEGFR-2 and VEGFR-3, and appears to associate as non-covalently linked dimers. Thus, preferred VEGF-D polynucleotides include those polynucleotides that comprise a nucleotide sequence encoding amino acids 93-201 of SEQ ID NO: 4, or comprising fragments thereof that retain VEGFR-3 and/or VEGFR-2 binding.

Moreover, since the therapeutic VEGF-D is to be administered as recombinant VEGF-D or indirectly via somatic gene therapy, it is within the skill in

the art (and an aspect of the invention) to make and use analogs of human VEGF-D (and polynucleotides that encode such analogs) wherein one or more amino acids have been added, deleted, or replaced with other amino acids, especially with conservative replacements, and wherein the VEGFR-3 binding activity has been retained. Analogs that retain VEGFR-3 binding biological activity are contemplated as VEGF-D polypeptides for use in the present invention. In a preferred embodiment, analogs having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 such modifications and that retain VEGFR-3 binding activity are contemplated as VEGF-D polypeptides for use in the present invention. Polynucleotides encoding such analogs are generated using conventional PCR, site-directed mutagenesis, and chemical synthesis techniques. Molecules that bind and stimulate phosphorylation of VEGFR-3 are preferred.

Preferred fragments of VEGF-C or -D for use in making the chimeric molecules of the invention are continuous fragments that bind VEGFR-3. However, it has been demonstrated that VEGFR-3 binding can be achieved with molecules that incorporate discrete, discontinuous fragments of VEGF-C, fused, e.g., to fragments of VEGF-A or other amino acid sequences. Such chimeric VEGFR-3 ligands are described in U.S. Patent Application Serial No. 09/795,006, filed February 26, 2001, and International Patent Publication No. WO 01/62942, each of which is incorporated herein by reference in its entirety. The methods and compositions described in these documents may be used in the present invention to produce VEGF-C chimeras having a heparin binding domain. Moreover, the same teachings also apply to using continuous or discontinuous fragments of VEGF-D to make molecules that bind VEGFR-3.

In still another variation, the VEGFR-3 ligand sequence for use in making chimeras of the invention is itself a chimeric molecule comprised of VEGF-C and VEGF-D sequences. The foregoing documents describe methods for making such chimeras and confirming their VEGFR-3 binding activity.

In addition to binding VEGFR-3, the VEGFR-3 binding domain used to make molecules of the invention optionally also binds VEGFR-2. In addition, the molecule optionally binds VEGFR-1 and/or one or more neuropilin molecules.

Receptor binding assays for determining the binding of such chimeric molecules to one or more of these receptors are well-known in the art. Examples of such receptor binding assays are taught in *e.g.*, U.S. Patent Application No. 09/795,006, and WO 01/62942, each incorporated herein by reference. (See, e.g., Example 3 of U.S. Patent Application No. 09/795,006, and WO 01/62942, which details binding assays of VEGF-C and related VEGF receptor ligands to soluble VEGF receptor-Fc fusion proteins. Example 5 of those documents details analysis of receptor activation or inhibition by such ligands. Example 6 describes analyses of receptor binding affinities of such ligands.) In addition, Achen *et al., Proc Natl Acad Sci USA 95*:548-53 (1998), incorporated by reference in its entirety, teaches exemplary binding assays. The binding of the chimeric VEGF polypeptides having the formula **X-B-Z** to any one or more of VEGF receptors, VEGFR-1, VEGFR-2, and VEGFR-3, may be analyzed using such exemplary assays.

Domain Z: a heparin binding domain

Domain Z of the chimeric **X-B-Z** molecules is any substance that possesses heparin binding activity and therefore confers such heparin binding activity to the chimeric polypeptide. Without being bound to any mechanisms of action, it is contemplated that the presence of a heparin binding domain on the growth factors facilitates the binding of the growth factors to heparin and allows the concentration of the growth factors in the extracellular matrix to increase the efficiency of binding of the growth factors to their respective cell surface receptors, thereby increasing the bioavailability of the growth factors at a given site.

VEGF-C and VEGF-D, like $VEGF_{121}$, lack a heparin binding domain. However, it is known that $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$, comprise heparin-binding domains (Keck *et al., Arch. Bioch. Biophys.*, 344:103-113, 1997; Fairbrother *et al., Structure* 6:637-648, 1998). Exons 6 (21 amino acids) and 7 (44 amino acids) contain two independent heparin binding domains (Poltorak *et al., Herz*, 25:126-9, 2000). In preferred aspects of the present invention, subunit **Z** is a heparin binding domain encoded by exon 6, and/or exon 7 of VEGF. Subunit **Z** may further comprise the amino acids encoded by exon 8 of VEGF. The sequences of the various exons of VEGF are widely known and may be found at *e.g.*, Genbank Accession

numbers M63976-M63978, where M63976 is exon 6 (SEQ ID NO: 9), M63977 is exon 7 (SEQ ID NO: 11); and M63978 is exon 8 (SEQ ID NO: 13).

As noted herein, the human VEGF-A gene is expressed as numerous isoforms, including $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$. A human $VEGF_{206}$ sequence obtained from the Swiss Prot database (accession no. P15692) is set forth below and in SEQ ID NO: 5:

```
1   mnfllswvhw slalllylhh akwsqaapma egggqnhhev vkfmdvyqrs ychpietlvd
61  ifqeypdeie yifkpscvpl mrcggccnde glecvptees nitmqimrik phqgqhigem
121 sflqhnkcec rpkkdrarqe kksvrgkgkg qkrkrkksry kswsvyvgar cclmpwslpg
181 phpcgpcser rkhlfvqdpq tckcsckntd srckarqlel nertcrcdkp rr
```

Amino acids 1-26 of this sequence represent the signal peptide and mature $VEGF_{206}$ comprises amino acids 27-232. Referring to the same sequence, the signal peptide and amino acids 142-226 are absent in mature isoform $VEGF_{121}$ (SEQ ID NO: 12). The signal peptide and amino acids 166-226 are absent in mature isoform $VEGF_{145}$ (SEQ ID NO: 14). The signal peptide and amino acids 142-182 are absent in mature isoform $VEGF_{165}$ (SEQ ID NOs: 6-7). The signal peptide and amino acids 160-182 are absent in mature isoform $VEGF_{183}$. The signal peptide and amino acids 166-182 are absent in mature isofrom $VEGF_{189}$.

Referring to Figure 1B and the foregoing sequence, amino acids 142-165 correspond to exon 6a (found in VEGF isoforms 145, 189, and 206); amino acids 166-182 correspond to exon 6b (found in isofrom 206 only); and amino acids 183-226 correspond to exon 7 (found in isoforms 165, 189, and 206).

Thus, referring again to the same sequence, the apparent heparin binding domain within $VEGF_{145}$ corresponds to amino acids 142-165 or a fragment thereof. The apparent heparin binding domain of $VEGF_{165}$ corresponds to amino acids 183-226 or a fragment thereof.

The apparent heparin binding domain(s) of $VEGF_{189}$ (SEQ ID NO: 10) correspond to amino acids 142-165 joined directly to amino acids 183-226, or fragment(s)s thereof. The apparent heparin binding domain(s) of $VEGF_{206}$ correspond to amino acids 142-226, or fragment(s) thereof.

In other embodiments, subunit **Z** may be derived from the heparin binding domains of other, non-VEGF growth factors. For example, subunit **Z** may be

the heparin binding domain of VEGF-B. Makinen *et al.*, (*J. Biol. Chem.*, 274:21217-22, 1999), have described various isoforms of VEGF-B and have shown that the exon 6B encoded sequence of VEGF-B_{167} resembles the heparin and NRP1-binding domain encoded by exon 7 of $VEGF_{165}$. Thus exon-6B of VEGF-B_{167} (or a heparin binding fragment thereof) may be used as the heparin binding subunit **Z** of the chimeric molecules of the present invention. The publication of Makinen *et al.*, *J. Biol. Chem.*, 274: 21217-22, 1999 provides a detailed description of the construction of the VEGF-B exon 6B-encoded sequence. Nucleotide and deduced amino acid sequences for VEGF-B are deposited in GenBank under Acc. No. U48801, incorporated herein by reference. Also incorporated herein by reference is Olofsson *et al., J. Biol. Chem.* 271 (32), 19310-19317 (1996), which describes the genomic organization of the mouse and human genes for VEGF-B, and its related Genbank entry at AF468110, which provides an exemplary genomic sequence of VEGF-B.

Mulloy *et al.*, (*Curr Opin Struct Biol.* 11(5):623-8, 2001) describes properties from many heparin binding domain structures and identifies many heparin binding domain examples, and is incorporated herein by reference. Any such heparin binding domains may be used in the chimeric molecules of the present invention. In still further embodiments, subunit Z may comprise the heparin binding domain of PlGF-2 (see Hauser and Weich, *Growth Factors,* 9 259-68, 1993). Heparin binding domains from other growth factors also may be used in the present chimeric polypeptides, such as for example the heparin binding domain from EGF-like growth factor (Shin *et al., J Pept Sci.* 9(4):244-50, 2003); the heparin binding domain from insulin-like growth factor-binding protein (Shand *et al., J Biol Chem.* 278(20):17859-66, 2003), and the like. Other heparin binding domains that may be used herein include, but are not limited to, the pleiotrophin and amphoterin heparin binding domains (*Matrix Biol.* 19(5):377-87, 2000); CAP37 (Heinzelmann *et al., Int J Surg Investig.* 2(6):457-66, 2001); and the heparin-binding fragment of fibronectin (Yasuda *et al., Arthritis Rheum.* 48(5):1271-80, 2003).

Those of skill in the art are aware that heparin binding domains are present on numerous other proteins, including e.g., apolipoprotein E (SEQ ID NO: 61, residues 162-165, 229-236), fibronectin (SEQ ID NO: 62), amphoterin (SEQ ID NO: 63), follistatin (SEQ ID NO: 64), LPL (SEQ ID NO: 65), myeloperoxidase (SEQ ID NO: 66), other growth factors, and the like. Merely by way of example, the protein

sequences of various heparin binding proteins found in Genbank include but are not limited to 1LR7_A; 1LR8_A; 1LR9_A; AAH05858 (FN1, SEQ ID NO: 58); NP_000032 (SEQ ID NO: 54); NP_000177 (H Factor 1, SEQ ID NO: 52); NP_001936 (dip theria toxin receptor, SEQ ID NO: 51); NP_002328 (alpha-2-MRAP, SEQ ID NO: 53); NP_005798 (proteoglycan 4, SEQ ID NO: 55); NP_009014 (SEQ ID NO: 36); NP_032018; NP_032511; NP_034545; NP_035047; NP_037077; NP_498403; NP_604447; NP_932158 (SEQ ID NO: 37); NP_990180; O15520 (SEQ ID NO: 50); O35565; O46647; P01008 (SEQ ID NO: 40); P02649 (SEQ ID NO: 35); P02749 (SEQ ID NO: 39); P02751 (SEQ ID NO: 59); P04196 (SEQ ID NO: 42); P04937; P05546 (SEQ ID NO: 56); P05770; P06858 (SEQ ID NO: 44); P07155; P07589; P08226; P10517; P11150 (SEQ ID NO: 43); P11276; P11722_1; P11722_2; P15656; P15692 (SEQ ID NO: 57); P17690; P18287; P18649; P18650; P20160 (SEQ ID NO: 38); P23529; P26644; P27656; P30533 (SEQ ID NO: 45); P33703; P35268 (SEQ ID NO: 49); P47776; P49182; P49763 (SEQ ID NO: 47); P51858 (SEQ ID NO: 41); P51859; P55031; P61150; P61328; P61329; Q01339; Q01580; Q06186; Q11142; Q15303; Q28275; Q28377; Q28502; Q28640; Q28995; Q61092; Q61851; Q64268; Q7M2U7; Q8VHK7; Q91740; Q95LB0; Q99075 (SEQ ID NO: 46); Q9GJU3; Q9WVG5; Q9Y5X9 (SEQ ID NO: 48); XP_357846; XP_357859; XP_358238; XP_358249; 1304205A (SEQ ID NO: 31); 1AE5 (SEQ ID NO: 30); 1B9Q_A; 1FNH_A (SEQ ID NO: 29); 1KMX_A (SEQ ID NO: 28); 1MKC_A; 1OKQ_A; A35969 (SEQ ID NO: 21); A38432 (SEQ ID NO: 22); A41178 (SEQ ID NO: 23); A41914; A48991; AAA37542; AAA50562 (SEQ ID NO: 34); AAA50563 (SEQ ID NO: 32).; AAA50564 (SEQ ID NO: 33); AAA81780; AAB27481; AAB33125; AAC42069; AAD29416; B40080; C40862 (SEQ ID NO: 60); I39383 (SEQ ID NO: 24); IB9P_A; JC1409; JC1410; JC4168; JT0573; LPHUB (SEQ ID NO: 25); LPHUE (SEQ ID NO: 26); O18739; O19113; P11151; P11153; P11602; P12034 (SEQ ID NO: 27); P13387; P41104; P48807; P49060; P49923; P55302; P70492; Q06000; Q06175; Q09118; Q11184; Q29524; Q91289; Q9CB42; Q9R1E9; S26049; S27162; S51242; XP_134550; XP_142078; XP_145641; XP_212881; XP_213021; XP_227645; XP_232701; XP_344685; XP_344947; XP_345821; XP_346046; XP_357159; XP_357228; XP_357258; XP_358223. In addition, the heparin binding domain may be one derived from any of these proteins. In exemplary embodiments heparin binding of the domain may be determined by e.g., heparin affinity chromatography. In alternative embodiments, the heparin binding domain may be

assessed using methods described in U.S. Patent Number 6,274,704. The heparin binding peptides described therein also may by useful.

Domain B: a covalent linkage between X and Z.

Within the chimeric molecules of the formula **X-B-Z,** the term **B** denotes a linkage, preferably a covalent linkage, between subunit **X** and subunit **Z.** In some embodiments, **B** simply denotes a covalent bond. For example, in a preferred embodiment, where **X-B-Z** comprises a single continuous polypeptide, **B** can denote an amide bond between the C-terminal amino acid of **X** and the N-terminal amino acid of **Z**, or between the C-terminal amino acid of **Z** and the N-terminal amino acid of **X**. Another way to describe such embodiments is by the simplified formulas **X-Z** or **Z-X.**

The linker may be an organic moiety constructed to contain an alkyl, aryl backbone and may contain an amide, ether, ester, hydrazone, disulphide linkage or any combination thereof. Linkages containing amino acid, ether and amide bound components will be stable under conditions of physiological pH, normally 7.4 in serum and 4-5 on uptake into cells (endosomes). Preferred linkages are linkages containing esters or hydrazones that are stable at serum pH but hydrolyse to release the drug when exposed to intracellular pH. Disulphide linkages are preferred because they are sensitive to reductive cleavage; amino acid linkers can be designed to be sensitive to cleavage by specific enzymes in the desired target organ. Exemplary linkers are set out in Blattler et al. Biochem. 24:1517-1524, 1985; King et al.. Biochem. 25:5774-5779, 1986; Srinivasachar and Nevill, Biochem. 28:2501-2509, 1989.

In still other embodiments, entity **B** is a chemically, or otherwise, cleavable bond that, under appropriate conditions, allows the release of subunit **X** from subunit **Z**. For example domains **X** and **Z** can be covalently linked by one or more disulfide bridges linking cysteine residues of **X** and **Z**; or by mutual attachment to a distinct chemical entity, such as a carbohydrate moiety.

In particular embodiments, entity **B** comprises a peptide linker comprising from 1 to about 500 amino acids in length. Linkers of 4-50 amino acids are preferred, and 4-15 are highly preferred. Preferred linkers are joined N-terminally

and C-terminally to domains **X** and **Z** so as to form a single continuous polypeptide. In certain embodiments, the peptide linker comprises a protease cleavage site selected from the group consisting of a Factor Xa cleavage site, an enterokinase cleavage site (New England Biolabs), a thrombin cleavage site, a TEV protease cleavage site (Life Technologies), and a PreScission cleavage site (Amersham Pharmacia Biotech). The presence of such cleavage sites between subunit **X** and subunit **Z** will allow for the efficient release of effective amounts of subunit **X** in a suitable proteolytic milieu.

Processing of VEGF-C and -D is believed to occur in part intracellularly, but processing of the amino terminal pro-peptide is believed to occur following secretion. Cleavage of this pro-peptide is apparently necessary for VEGFR-2-mediated activity. In one variation of the invention, subunit **B** comprises an amino acid sequence analogous to the VEGF-C or -D N-terminal pro-peptide processing site, to make subunits **X** and **Z** susceptible to cleavage by the same protease that process these N-terminal pro-pepticles *in vivo*.

For example, with respect to VEGF-C, propeptide cleavage can occur at about amino acids 102/103 of SEQ ID NO: 2, and a suitable subunit **B** optionally include about 3-30 amino acids upstream and downstream of this site. The analogous processing site of VEGF-D occurs between residues 92 and 93 of SEQ ID NO: 4.

The linker is optionally a heterologous protein polypeptide. The linker may affect whether the polypeptide(s) to which it is fused to is able to dimerize to each other or to another polypeptide. Other chemical linkers are possible, as the linker need not be in the form of a polypeptide. However, when the linker comprises a peptide, the binding construct (with linker) allows for expression as a single molecule. Linker may be chosen such that they are less likely to induce an allergic or antigenic reaction.

More than one linker may be used per molecule of X-B-Z or Z-B-X. The linker may be selected for optimal conformational (steric) freedom between the growth factor and heparin binding domains allow them to interact with binding partners. The linker may be linear such that **X** and **Z** are linked in series, or the linker may serve as a scaffold to which two or more **X** or **Z** binding units are attached. A linker may also have multiple branches. For example, using linkers disclosed in Tam, *J. Immunol. Methods* 196:17 (1996). **X** or **Z** domains may be attached to each other

or to the linker scaffold via N-terminal amino groups, C-terminal carboxyl groups, side chains, chemically modified groups, side chains, or other means.

When comprising peptides, the linker may be designed to have sequences that permit desired characteristics. For example, the use of glycyl residues allow for a relatively large degree of conformational freedom, whereas a proline would tend to have the opposite effect. Peptide linkers may be chosen so that they achieve particular secondary and tertiary structures, *e.g.*, alpha helices, beta sheets and beta barrels. Quarternary structure can also be utilized to create linkers that join two binding units together non-covalently. For example, fusing a protein domain with a hydrophobic face to each binding unit may permit the joining of the two binding units via the interaction between the hydrophobic interaction of the two molecules. In some embodiments, the linker may provide for polar interactions. For example, a leucine zipper domain of the proto-oncoproteins Myc and Max, respectively may be used. Luscher and Larsson, Ongogene 18:2955-2966 (1999). In some embodiments, the linker allows for the formation of a salt bridge or disulfide bond. Linkers may comprise non-naturally occurring amino acids, as well as naturally occurring amino acids that are not naturally incorporated into a polypeptide. In some embodiments, the linker comprises a coordination complex between a metal or other ions and various residues from the multiple peptides joined thereby.

Linear peptide linkers may have various lengths, and generally consist of at least one amino acid residue. In some embodiments the linker has from 1 to 10 residues. In some embodiments, the linker has from 1 to 50 residues. In some embodiments, the linker has from 1-100 residues. In some embodiments, the linker has from 1-1000 residues. In some embodiments the linker has 1-10,000 residues. In some embodiments the linker has more than 10,000 residues. In some embodiments, the linear peptide linker comprises residues with relatively inert side chains. Peptide linker amino acid residues need not be linked entirely or at all via alpha-carboxy and alpha-amino groups. That is, peptides may be linked via side chain groups of various residues. In some embodiments, a linker is used as is described in Liu et al. U.S. Pat. Appl. Pub. No. 2003/0064053.

B. Methods of Making Chimeric VEGF polypeptides

The chimeric molecules of the invention can be synthesized in solution or on a solid support in accordance with conventional techniques. Such polypeptides may be synthesized as small fragments of the complete chimeric polypeptide or as a complete full length sequence. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2d. ed., Pierce Chemical Co., (1984);Tam *et al.*, *J. Am. Chem. Soc.*, 105:6442, (1983); Merrifield, *Science*, 232: 341-347, (1986); and Barany and Merrifield, The Peptides, Gross and Meienhofer, eds, Academic Press, New York, 1-284, (1979), each incorporated herein by reference. The chimeric VEGF polypeptides of the invention having the formula **X-B-Z** or **Z-B-X**, can be readily synthesized and then screened using any of a number of assays that identify the polypeptides for VEGF-C-like, VEGF-D-like or other VEGF-like activity, such as *e.g.*, binding to VEGFR-1, VEGFR-2, or VEGFR-3, induction of vascular permeability, activity in an endothelial cell proliferation assay, induction of growth of lymphatic vessels, promotion of growth and differentiation of CD34+progenitor cells *in vitro*, activity in CAM assays, and the like. These and other assays for determining the activity of the vascular endothelial growth factor activity are described in U.S. Patent Application No. 09/795,006, and WO 01/62942.

Examples of solid-phase technology that may be used in the present invention include a Model 433A from Applied Biosystems Inc peptide synthesizer. Methods of using such automated solid phase synthesizers to produce pure polypeptides are well known.

As an alternative to automated peptide synthesis, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a chimeric polypeptide of the invention is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. Recombinant methods are especially preferred for producing longer polypeptides that comprise peptide sequences of the invention. Chimeric molecules of the invention also may be produced by a combination of techniques whereby domains are synthesized recombinantly or synthetically in two or more steps and joined together as a single polypeptide.

A variety of expression vector/host systems may be utilized to contain and express the chimeric polypeptide coding sequence. These include but are not

limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transfected with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmid); or animal cell systems. Mammalian cells that are useful in recombinant protein productions include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells. Exemplary protocols for the recombinant expression of the polypeptides in bacteria, yeast and other invertebrates are described herein below.

Expression vectors for use in prokaryotic hosts generally comprise one or more phenotypic selectable marker genes. Such genes generally encode, *e.g.*, a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors (LTI, Bethesda, MD), Bluescript vectors (Stratagene), pET vectors (Novagen) and pQE vectors (Qiagen). The DNA sequence encoding a peptide domain or chimeric polypeptide is cloned into such a vector, for example, pGEX-3X (Pharmacia, Piscataway, NJ) designed to produce a fusion protein comprising glutathione-S-transferase (GST), encoded by the vector, and a protein encoded by a DNA fragment inserted into the vector's cloning site. Treatment of the recombinant fusion protein with thrombin or factor Xa (Pharmacia, Piscataway, NJ) is expected to cleave the fusion protein, releasing the polypeptide of interest from the GST portion. The pGEX-3X/chimeric VEGF polypeptide construct is transformed into *E. coli* XL-1 Blue cells (Stratagene, La Jolla CA), and individual transformants were isolated and grown. Plasmid DNA from individual transformants is purified and partially sequenced using an automated sequencer to confirm the presence of the desired peptide or polypeptide encoding nucleic acid insert in the proper orientation.

Induction of the GST/substrate fusion protein is achieved by growing the transformed XL-1 Blue culture at 37°C in LB medium (supplemented with carbenicillin) to an optical density at wavelength 600 nm of 0.4, followed by further

incubation for 4 hours in the presence of 0.5 mM Isopropyl β-D-Thiogalactopyranoside (Sigma Chemical Co., St. Louis MO).

The GST fusion protein, expected to be produced as an insoluble inclusion body in the bacteria, may be purified as follows. Cells are harvested by centrifugation; washed in 0.15 M NaCl, 10 mM Tris, pH 8, 1 mM EDTA; and treated with 0.1 mg/ml lysozyme (Sigma Chemical Co.) for 15 minutes at room temperature. The lysate is cleared by sonication, and cell debris is pelleted by centrifugation for 10 minutes at 12,000 X g. The fusion protein-containing pellet is resuspended in 50 mM Tris, pH 8, and 10 mM EDTA, layered over 50% glycerol, and centrifuged for 30 min. at 6000 X g. The pellet is resuspended in standard phosphate buffered saline solution (PBS) free of Mg^{++} and Ca^{++}. The fusion protein is further purified by fractionating the resuspended pellet in a denaturing SDS polyacrylamide gel (Sambrook *et al.*, *supra*). The gel is soaked in 0.4 M KCl to visualize the protein, which is excised and electroeluted in gel-running buffer lacking SDS. If the GST/chimeric VEGF polypeptide fusion protein is produced in bacteria as a soluble protein, it may be purified using the GST Purification Module (Pharmacia Biotech).

The fusion protein may be subjected to thrombin digestion to cleave the GST from the chimeric VEGF polypeptide. The digestion reaction (20-40 μg fusion protein, 20-30 units human thrombin (4000 U/mg (Sigma) in 0.5 ml PBS) is incubated 16-48 hrs. at room temperature and loaded on a denaturing SDS-PAGE gel to fractionate the reaction products. The gel is soaked in 0.4 M KCl to visualize the protein bands. The identity of the protein band corresponding to the expected molecular weight of the chimeric VEGF polypeptide may be confirmed by partial amino acid sequence analysis using an automated sequencer (Applied Biosystems Model 473A, Foster City, CA).

Alternatively, the DNA sequence encoding the predicted substrate containing fusion polypeptide may be cloned into a plasmid containing a desired promoter and, optionally, a leader sequence (see, e.g., Better *et al.*, *Science*, 240: 1041 43, 1988). The sequence of this construct may be confirmed by automated sequencing. The plasmid is then transformed into *E. coli* using standard procedures employing $CaCl_2$ incubation and heat shock treatment of the bacteria (Sambrook *et al.*, *supra*). The transformed bacteria are grown in LB medium supplemented with carbenicillin, and production of the expressed protein is induced by growth in a

suitable medium. If present, the leader sequence will effect secretion of the chimeric VEGF polypeptide and be cleaved during secretion. The secreted recombinant protein may then be purified using conventional protein purification techniques.

Similarly, yeast host cells from genera including *Saccharomyces*, *Pichia*, and *Kluveromyces* may be employed to generate the peptide recombinantly. Preferred yeast hosts are *S. cerevisiae* and *P. pastoris*. Yeast vectors will often contain an origin of replication sequence from a 2T yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Vectors replicable in both yeast and *E. coli* (termed shuttle vectors) may also be used. In addition to the above-mentioned features of yeast vectors, a shuttle vector will also include sequences for replication and selection in *E. coli*. Direct secretion of polypeptides expressed in yeast hosts may be accomplished by the inclusion of nucleotide sequence encoding the yeast I-factor leader sequence at the 5' end of the substrate-encoding nucleotide sequence.

Generally, a polypeptide is recombinantly expressed in yeast using a commercially available expression system, e.g., the Pichia Expression System (Invitrogen, San Diego, CA), following the manufacturer's instructions. This system also relies on the pre pro alpha sequence to direct secretion, but transcription of the insert is driven by the alcohol oxidase (AOX1) promoter upon induction by methanol.

The secreted recombinant substrate is purified from the yeast growth medium by, e.g., the methods used to purify substrate from bacterial and mammalian cell supernatants.

Alternatively, the chimeric VEGF polypeptide may be expressed in an insect system. Insect systems for protein expression are well known. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The polypeptide coding sequence is cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of substrate will render the polyhedrin gene inactive and produce recombinant virus lacking protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the substrate is

expressed (Smith *et al., J Virol* 46: 584, 1983; Engelhard EK *et al., Proc Nat Acad Sci* 91: 3224-7, 1994). For example, DNA encoding a polypeptide of the invention may be cloned into the baculovirus expression vector pVL1393 (PharMingen, San Diego, CA; Luckow and Summers, Bio/Technology 6:47 (1988)). This resulting vector is then used according to the manufacturer's directions (PharMingen) to infect *Spodoptera frugiperda* cells in SF9 protein free media and to produce recombinant protein. The protein or peptide is purified and concentrated from the media using a heparin Sepharose column (Pharmacia, Piscataway, NJ) and sequential molecular sizing columns (Amicon, Beverly, MA), and resuspended in PBS. SDS PAGE analysis shows a single band and confirms the size of the protein, and Edman sequencing on a Porton 2090 Peptide Sequencer confirms its N terminal sequence.

Mammalian host systems for the expression of recombinant proteins also are well known. Host cell strains may be chosen for a particular ability to process the expressed protein or produce certain post translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, and the like have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

It is preferable that the transformed cells are used for long-term, high-yield protein production and as such stable expression is desirable. Once such cells are transformed with vectors that contain selectable markers along with the desired expression cassette, the cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The selectable marker is designed to confer resistance to selection and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell.

A number of selection systems may be used to recover the cells that have been transformed for recombinant protein production. Such selection systems

include, but are not limited to, HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk-, hgprt- or aprt- cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for dhfr, that confers resistance to methotrexate; gpt, that confers resistance to mycophenolic acid; neo, that confers resistance to the aminoglycoside G418; als which confers resistance to chlorsulfuron; and hygro, that confers resistance to hygromycin. Additional selectable genes that may be used include trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine. Markers that give a visual indication for identification of transformants include anthocyanins, b-glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin.

Protein Purification.

It will be desirable to purify the chimeric VEGF polypeptide of the present invention. Protein purification techniques are well known. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the peptide or polypeptides of the invention from other proteins, the polypeptides or peptides of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity).

Generally, "purified" will refer to a polypeptide, protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the polypeptide, protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Various methods for quantifying the degree of purification of the polypeptide, protein or peptide will be apparent. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to

compare it to the specific activity of the initial extract, and to thus calculate the degree of purity, herein assessed by a "-fold purification number." The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed polypeptide, protein or peptide exhibits a detectable activity.

Various techniques known for use in protein purification are also suitable for molecules of the present invention. These include, for example, precipitation with ammonium sulphate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite, exclusion, and affinity chromatography; isoelectric focusing; gel electrophoresis (including polyacrylamide gel electrophoresis); and combinations of such and other techniques. The order of conducting the various purification steps may be varied, and certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified polypeptide, protein or peptide.

There is no general requirement that the polypeptide, protein or peptide always be provided in their most purified state. Indeed, it is contemplated that less substantially purified products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater "-fold" purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al., Biochem. Biophys. Res. Comm.*, 76:425, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

In still another related embodiment, the invention provides a method for producing a vascular endothelial growth factor receptor binding protein,

comprising the steps of growing a host cell of the invention in a nutrient medium and isolating the polypeptide or variant thereof from the cell or the medium. Isolation of the polypeptide from the cells or from the medium in which the cells are grown is accomplished by purification methods known in the art, *e.g.*, conventional chromatographic methods including immunoaffinity chromatography, receptor affinity chromatography, hydrophobic interaction chromatography, lectin affinity chromatography, size exclusion filtration, cation or anion exchange chromatography, high pressure liquid chromatography (HPLC), reverse phase HPLC, and the like. Still other methods of purification include those wherein the desired protein is expressed and purified as a fusion protein having a specific tag, label, or chelating moiety that is recognized by a specific binding partner or agent. The purified protein can be cleaved to yield the desired protein, or be left as an intact fusion protein. Cleavage of the fusion component may produce a form of the desired protein having additional amino acid residues as a result of the cleavage process.

In preferred embodiments, purification of the chimeric polypeptides of the present invention may be achieved using affinity purification using an extracellular domain of Flt4, or other portion of a receptor that the chimeric polypeptides of the invention may bind. Exemplary affinity purification of VEGF-C related compositions is described in e.g., U.S. Patent No. 5,776,755, incorporated herein by reference. In an exemplary affinity purification procedure using the FLT4 extracellular domain, the chimeric polypeptide-containing composition to be purified are initially concentrated 30-50 fold using Centriprep filter cartridges and loaded onto a column of immobilized FLT4 extracellular domain. Two affinity matrices are prepared. In the first case, the Flt4EC-6xHis fusion protein is crosslinked to CNBr-activated Sepharose 4B (Pharmacia) and in the second case the FLT4-Ig fusion protein is coupled to protein A Sepharose using dimethylpimelidate (Schneider *et al.*, *J. Biol. Chem.* 257: 10766-10769, 1982). The material eluted from the affinity column is subjected to further purification using ion exchange and reverse-phase high pressure chromatography and SDS-polyacrylamide gel electrophoresis.

As the chimeric polypeptides of the present invention have the ability to bind VEGFR-3 and have the ability to bind heparin, one method of obtaining a highly purified specimen would be to subject the chimeric polypeptides to two types of affinity purification. One affinity purification being based on VEGFR-3 binding

property of the chimeric polypeptides and the second affinity purification being based on the heparin binding property of the chimeric polypeptides. Heparin-based affinity chromatography methods are well known. For example, one uses a commercially available heparin-Sepharose affinity chromatography system such as e.g., Heparin Sepharose™ 6 Fast Flow available from Amersham Biosciences (Piscataway, NJ). Heparin Sepharose also is available from Pharmacia (Uppsula, Sweden). Other heparin affinity chromatography resins are available from Sigma Aldrich (St. Louis, MO). Exemplary protocols for purifying VEGF165 using Heparin-Sepharose CL6B affinity chromatography are presented by Ma *et al.*, (*Biomed Environ Sci.* 14(4):302-11, 2001), Dougher *et al.*, (*Growth Factors,* 14(4):257-68, 1997). Such methods could be used for the purification of the chimeric polypeptides of the present invention. Where these methods are used in conjunction with the FLT4 receptor-based affinity purification discussed above, the receptor-based affinity purification may be performed before or after the heparin binding affinity chromatography step.

Yet another affinity chromatography purification procedure that may be used to purify the chimeric polypeptides of the present invention employs immunoaffinity chromatography using antibodies specific for either the heparin binding domain of the chimeric polypeptides or more preferably antibodies specific for the domain X of the chimeric polypeptides. Antibodies specific for domain X would be any antibodies that are specific for VEGF-C, VEGF-D or chimeras of VEGF-D. In addition, purification of the chimeric polypeptides of the present invention may be achieved using methods for the purification of VEGF-C or VEGF-D that are described in U.S. Patent No. 6,361,946 and WO 98/07832, respectively.

C. Nucleic Acids and Related Compositions.

The invention embraces polynucleotides that encode the chimeric VEGF polypeptides discussed above and also polynucleotides that hybridize under moderately stringent or high stringency conditions to the complete non-coding strand, or complement, of such polynucleotides. Due to the well-known degeneracy of the universal genetic code, one can synthesize numerous polynucleotide sequences that encode each chimeric polypeptide of the present invention. All such polynucleotides are contemplated to be useful in the present application. Particularly preferred polynucleotides join a natural human VEGFR-3 receptor ligand cDNA sequence *e.g.*,

a sequence of SEQ ID NO:1 or SEQ ID NO:3, preferably a fragment thereof encoding a VEGFR-3 binding domain, with a natural human heparin binding domain encoding sequence. This genus of polynucleotides embraces polynucleotides that encode polypeptides with one or a few amino acid differences (additions, insertions, or deletions) relative to amino acid sequences specifically taught herein. Such changes are easily introduced by performing site directed mutagenesis, for example.

One genus of both polynucleotides of the invention and polypeptides encoded thereby can be defined by molecules with a first domain that hybridize under specified conditions to a VEGF-C or -D polynucleotide sequence and a second domain that hybridizes under the same conditions to naturally occurring human sequences that encode heparin binding domains taught herein.

Exemplary highly stringent hybridization conditions are as follows: hybridization at 65°C for at least 12 hours in a hybridization solution comprising 5X SSPE, 5X Denhardt's, 0.5% SDS, and 2 mg sonicated non homologous DNA per 100 ml of hybridization solution; washing twice for 10 minutes at room temperature in a wash solution comprising 2X SSPE and 0.1% SDS; followed by washing once for 15 minutes at 65°C with 2X SSPE and 0.1% SDS; followed by a final wash for 10 minutes at 65°C with 0.1X SSPE and 0.1% SDS. Moderate stringency washes can be achieved by washing with 0.5X SSPE instead of 0.1X SSPE in the final 10 minute wash at 65°C. Low stringency washes can be achieved by using 1X SSPE for the 15 minute wash at 65°C, and omitting the final 10 minute wash. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, *et al.* (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook *et al.*, (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51. For example, the invention provides a polynucleotide that comprises a nucleotide sequence that hybridizes under moderately stringent or high stringency hybridization conditions to any specific nucleotide sequence of the invention, and that encodes a chimeric polypeptide as described herein that binds at least one of the naturally

occurring vascular endothelial growth factor or platelet derived growth factor receptors.

In a related embodiment, the invention provides a polynucleotide that comprises a nucleotide sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to any specific nucleotide sequence of the invention, and that encodes a polypeptide that binds heparin and at least one of the naturally occurring vascular endothelial growth factor or platelet derived growth factor receptors.

In a related embodiment, the invention provides vectors comprising a polynucleotide of the invention. Such vectors are useful, e.g., for amplifying the polynucleotides in host cells to create useful quantities thereof. In preferred embodiments, the vector is an expression vector wherein the polynucleotide of the invention is operatively linked to a polynucleotide comprising an expression control sequence. Autonomously replicating recombinant expression constructs such as plasmid and viral DNA vectors incorporating polynucleotides of the invention are specifically contemplated. Expression control DNA sequences include promoters, enhancers, and operators, and are generally selected based on the expression systems in which the expression construct is to be utilized. Preferred promoter and enhancer sequences are generally selected for the ability to increase gene expression, while operator sequences are generally selected for the ability to regulate gene expression. Expression vectors are useful for recombinant production of polypeptides of the invention. Expression constructs of the invention may also include sequences encoding one or more selectable markers that permit identification of host cells bearing the construct. Expression constructs may also include sequences that facilitate, and preferably promote, homologous recombination in a host cell. Preferred constructs of the invention also include sequences necessary for replication in a host cell.

Vectors also are useful for "gene therapy" treatment regimens, wherein a polynucleotide that encodes a polypeptide of the invention is introduced into a subject in need of treatment involving the modulation (stimulation or blockage) of vascular endothelial growth factor receptors, in a form that causes cells in the subject to express the polypeptide of the invention *in vivo*. Gene therapy aspects that are described in *e.g.*, U.S. Patent Application No. 09/795,006, and WO 01/62942, also are applicable herein.

In another related embodiment, the invention provides host cells, including prokaryotic and eukaryotic cells, that are transformed or transfected (stably or transiently) with polynucleotides of the invention or vectors of the invention. Polynucleotides of the invention may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein coding region or a viral vector. Methods for introducing DNA into the host cell, which are well known and routinely practiced in the art include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. As stated above, such host cells are useful for amplifying the polynucleotides and also for expressing the polypeptides of the invention encoded by the polynucleotide. Such host cells are useful in assays as described herein. For expression of polypeptides of the invention, any host cell is acceptable, including but not limited to bacterial, yeast, plant, invertebrate (e.g., insect), vertebrate, and mammalian host cells. For developing therapeutic preparations, expression in mammalian cell lines, especially human cell lines, is preferred. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., glycosylation, truncation, lipidation, and phosphorylation) as may be desirable to confer optimal biological activity on recombinant expression products of the invention. Glycosylated and non-glycosylated forms of polypeptides are embraced by the present invention. Similarly, the invention further embraces polypeptides described above that have been covalently modified to include one or more water soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol.

Also within the scope of the invention are compositions comprising polypeptides or polynucleotides of the invention. In a preferred embodiment, such compositions comprise one or more polynucleotides or polypeptides of the invention that have been formulated with a pharmaceutically acceptable (e.g., sterile and non toxic) diluent or carrier. Liquid, semisolid, or solid diluents that serve as pharmaceutical vehicles, excipients, or media are preferred. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, water, saline solutions, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl and propylhydroxybenzoate, talc, alginates, starches, lactose, sucrose, dextrose, sorbitol, mannitol, glycerol, calcium phosphate, mineral oil, and cocoa butter. Such

formulations are useful, e.g., for administration of polypeptides or polynucleotides of the invention to mammalian (including human) subjects in therapeutic regimens.

Similarly, the invention provides for the use of polypeptides or polynucleotides of the invention in the manufacture of a medicament for the treatment of disorders described herein, including but not limited to disorders characterized by insufficient or undesirable endothelial cell proliferation and/or disorders characterized by ischemia and/or vessel occlusion, wherein neovascularization is desirable.

In a related embodiment, the invention provides a kit comprising a polynucleotide, polypeptide, or composition of the invention packaged in a container, such as a vial or bottle, and further comprising a label attached to or packaged with the container, the label describing the contents of the container and providing indications and/or instructions regarding use of the contents of the container to treat one or more disease states as described herein.

In yet another aspect, the present invention provides methods of producing polypeptides having novel VEGF receptor binding and stimulation properties, and methods for producing polynucleotides that encodes such polypeptides.

As used herein, "modulate the growth of mammalian endothelial cells" means stimulate such growth by inducing a mitogenic signal through binding cell surface receptors expressed on vascular endothelial cells, or inhibiting such growth. The inhibition may be due to blockage of vascular or lymphatic endothelial growth factor receptors, or the formation of heterodimers with endogenous growth factors that prevent stimulation of endogenous receptors by the endogenous growth factors. Inhibition also may be achieved by conjugating cytotoxic agents to polypeptides of the invention that bind VEGF receptors. Exemplary toxins are known in the art and described elsewhere herein. Polypeptides of the invention conjugated to cytotoxic agents or other agents that modulate cell growth are contemplated as another aspect of the invention. Agonist molecules of the invention that stimulate endothelial cell growth are a preferred class of agents. Antagonists that inhibit endothelial cell growth also are preferred.

D. Methods of Using Chimeric VEGF-C and -D polypeptides

In yet another embodiment, the invention provides numerous *in vitro* and *in vivo* methods of using the chimeric polypeptides and polynucleotides of the invention. Generally speaking, the chimeric polypeptides of the invention are useful for modulating (stimulating or inhibiting) cellular processes that are mediated through any of the PDGF/VEGF family of receptors, such as for example, VEGFR-1, more preferably, VEGFR-2, and/or VEGFR-3. These receptors may be involved singularly in certain processes and in combination, to varying extents, in other processes. The chimeric polypeptides of the invention advantageously have a heparin binding domain which increases the potency of the VEGFR ligand in the biological processes in which it is involved.

Thus, in one variation, the invention provides a method of modulating the signaling of one or more of receptors for which either VEGF-C or VEGF-D are ligands. The method generally involves the step of contacting a cell that expresses such a receptor, *e.g.*, VEGFR-2, and/or VEGFR-3 with a composition comprising a chimeric polypeptide of the invention. In one variation, modulation to activate signaling is contemplated, and the cell is contacted with a polypeptide of the invention that stimulates receptor signaling in an amount sufficient to bind to the one or more receptors and induce receptor signaling. Preferably, an amount is employed that is effective to stimulate a cellular response such as an in vitro or in vivo endothelial cell proliferation and/or recruitment or angiogenesis or lymphangiogenesis.

In another variation, modulation to inhibit signaling is contemplated. The cell is contacted with a polypeptide that inhibits ligand-induced receptor activation (or a polypeptide conjugated to a cytotoxin), in an amount sufficient to inhibit signaling that is induced by receptor ligand growth factor polypeptides that exist endogenously in the cell's environment. Dose-response studies permit accurate determination of a proper quantity of chimeric polypeptide to employ. Effective quantities can be estimated from measurements of the binding affinity of a polypeptide for a target receptor, of the quantity of receptor present on target cells, of the expected dilution volume (*e.g.,* patient weight and blood volume for *in vivo* embodiments), and of polypeptide clearance rates. Existing literature regarding dosing of known VEGFR ligands also provides guidance for dosing of molecules of the invention.

In another variation, the invention provides a method of modulating the signaling of one or more of the receptors of VEGF-C or VEGF-D *in vivo*, comprising the step or administering to a mammalian subject in need of modulation of the signaling of one or more of these receptors a composition comprising a polynucleotide of the invention, under conditions in which cells of the subject are transformed or transfected by the polynucleotide and express the chimeric polypeptide of the invention encoded thereby, wherein the expressed chimeric polypeptide modulates signaling of the one or more receptors. Human subjects are preferred. Administering to subjects in need of therapy for conditions that will benefit from modulation of VEGFR receptors, are particularly contemplated.

Polypeptides of the present invention that bind and inhibit VEGFR-3 or that are conjugated to a cytotoxic moiety can be used to target neoplasia characterized by cells expressing VEGFR-3 on their surfaces.

Polypeptides of the invention that can activate VEGFR-3 can be used to promote the endothelial functions of lymphatic vessels and tissues such as to treat loss of lymphatic vessels, occlusions of lymphatic vessels, lymphangiomas, and primary idiopathic lymphedemas, including Milroy's disease and lymphedema praecox, as well as secondary lymphedemas, including those resulting from removal of lymph nodes and vessels, radiotherapy and surgery in treatment of cancer, trauma and infection.

Polynucleotides or polypeptides of the invention can be administered purely as a prophylactic treatment to prevent lymphedema in subjects at risk for developing lymphedema, or as a therapeutic treatment to subjects afflicted with lymphedema, for the purpose of ameliorating its symptoms (*e.g.*, swelling due to the accumulation of lymph).

The polynucleotides and polypeptides of the invention that activate VEGFR-3 can also be used to promote re-growth or permeability of lymphatic vessels in patients whose axillary lymphatic vessels were removed during surgical interventions in the treatment of cancer (e.g., breast cancer). Polynucleotides and polypeptides of the invention can be used to treat vascularization in, for example, organ transplant patients. A composition containing the polypeptide(s) of the invention may be directly applied to the isolated vessel segment prior to its being

grafted *in vivo* to minimize rejection of the transplanted material and to stimulate vascularization of the transplanted materials.

Polypeptides of the invention that activate VEGF receptor activity may be used to treat wounds, surgical incisions, sores, and other indications where healing is reasonably expected to be promoted if the process of neovascularization can be induced and/or accelerated. In certain embodiments, such polypeptides can be used to improve healing of skin flaps or skin grafts following surgery as described in commonly owned, co-filed U.S. Patent Application No. 60/478,114 (Filed June 12, 2003, attorney docket No. 28967/39117), and U.S. Patent Application No. _____ (attorney docket No. 28967/39117A), filed June 14, 2004, each incorporated herein by reference.

In addition, the expression of receptors for vascular endothelial growth factors have been observed in certain progenitor cells, such as hematopoietic and/or endothelial progenitor cells, and VEGF-C has been observed to have myelopoietic activity. These observations provide an indication that polynucleotides or polypeptides according to the invention may be used to treat or prevent inflammation, infection, or immune disorders by modulating the proliferation, differentiation and maturation, or migration of immune cells or hematopoietic cells. Polynucleotides or polypeptides according to the invention may also be useful to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels and migration in and out of the thymus. See International Patent Publication No. WO 98/33917, incorporated by reference.

Polynucleotides and polypeptides of the invention can be used for stimulating myelopoiesis (especially growth of neutrophilic granuloctyes) or inhibiting it. See International Patent Publication No. WO 98/33917, incorporated by reference. Thus, the invention includes a method for modulating myelopoiesis in a mammalian subject comprising administering to a mammalian subject in need of modulation of myelopoiesis an amount of a polypeptide of the invention that is effective to modulate myelopoiesis. In one embodiment, a mammalian subject suffering from granulocytopenia is selected, and the method comprises administering to the subject an amount of a polypeptide effective to stimulate myelopoiesis. In particular, a polypeptide of the invention is administered in an amount effective to increase the neutrophil count in blood of the subject.

In a related embodiment, the invention includes a method of increasing the number of neutrophils in the blood of a mammalian subject comprising the step of expressing in a cell in a subject in need of an increased number of blood neutrophils a DNA encoding a polynucleotide of the invention that is able to activate signaling through VEGF receptors, the DNA operatively linked to a promoter or other control sequence that promotes expression of the DNA in the cell. Similarly, the invention includes a method of modulating the growth of neutrophilic granulocytes *in vitro* or *in vivo* comprising the step of contacting mammalian stem cells with a polypeptide of the invention in an amount effective to modulate the growth of mammalian endothelial cells.

The invention also includes a method for modulating the growth of CD34+ progenitor cells (especially hematopoietic progenitor cells and endothelial progenitor cells, more preferably CD34+/VEGFR-3+, still more preferably CD34+, CD133+/VEGFR3+ cells) *in vitro* or *in vivo* comprising the step of contacting mammalian CD34+ progenitor cells with a polypeptide of the invention in an amount effective to modulate the growth of mammalian endothelial cells. For *in vitro* methods, CD34+ progenitor cells isolated from cord blood or bone marrow are specifically contemplated. Further isolation of the CD133+ VEGFR-3+ subfraction also is contemplated. *In vitro* and *in vivo* methods of the invention for stimulating the growth of CD34+ precursor cells also include methods wherein polypeptides of the invention are employed together (simultaneously or sequentially) with other polypeptide factors for the purpose of modulating hematopoiesis/myelopoiesis or endothelial cell proliferation. Such other factors include, but are not limited to colony stimulating factors ("CSFs," e.g., granulocyte-CSF (G-CSF), macrophage-CSF (M-CSF), and granulocyte-macrophage-CSF (GM-CSF)), interleukin-3 (IL-3, also called multi-colony stimulating factor), other interleukins, stem cell factor (SCF), other polypeptide factors, and their analogs that have been described and are known in the art. See generally *The Cytokine Handbook, Second Ed.,* Angus Thomson (editor), Academic Press (1996); Callard and Gearing, *The Cytokine FactsBook*, Academic Press Inc. (1994); and Cowling and Dexter, *TIBTECH, 10(10)*:349-357 (1992). The use of a polypeptide of the invention as a progenitor cell or myelopoietic cell growth factor or co-factor with one or more of the foregoing factors may potentiate previously unattainable myelopoietic effects and/or potentiate previously attainable

myelopoietic effects while using less of the foregoing factors than would be necessary in the absence of a polypeptide of the invention.

Polynucleotides and polypeptides of the invention may also be used in the treatment of lung disorders to improve blood circulation in the lung and/or gaseous exchange between the lungs and the blood stream; to improve blood circulation to the heart and O_2 gas permeability in cases of cardiac insufficiency; to improve blood flow and gaseous exchange in chronic obstructive airway disease; and to treat conditions such as congestive heart failure, involving accumulations of fluid in, for example, the lung resulting from increases in vascular permeability, by exerting an offsetting effect on vascular permeability in order to counteract the fluid accumulation.

Polypeptides of the invention that bind but do not stimulate signaling through one or more of the VEGF receptors may be used to treat chronic inflammation caused by increased vascular permeability, retinopathy associated with diabetes, rheumatoid arthritis and psoriasis. Polynucleotides or polypeptides according to the invention that are able to inhibit the function of one or more VEGF receptors can also be used to treat edema, peripheral arterial disease, Kaposi's sarcoma, or abnormal retinal development in premature newborns.

In another embodiment, the invention provides a method for modulating the growth of endothelial cells in a mammalian subject comprising the steps of exposing mammalian endothelial cells to a polypeptide according to the invention in an amount effective to modulate the growth of the mammalian endothelial cells. In one embodiment, the modulation of growth is affected by using a polypeptide capable of stimulating tyrosine phosphorylation of VEGF receptors in a host cell expressing the VEGF receptors. In modulating the growth of endothelial cells, the invention contemplates the modulation of endothelial cell-related disorders. In a preferred embodiment, the subject, and endothelial cells, are human. The endothelial cells may be provided *in vitro* or *in vivo*, and they may be contained in a tissue graft. An effective amount of a polypeptide is an amount necessary to achieve a reproducible change in cell growth rate (as determined by microscopic or macroscopic visualization and estimation of cell doubling time, or nucleic acid synthesis assays).

Since angiogenesis and neovascularization are essential for tumor growth, inhibition of angiogenic activity can prevent further growth and even lead to regression of solid tumors. Likewise inhibition of lymphangeogenesis may be instrumental in preventing metastases. See e.g., International Publication Nos. WO 02/060950 and WO 00/21560, incorporated herein by reference. Polynucleotides and polypeptides of the invention, when conjugated to a cytotoxic agent may be used to treat neoplasias including sarcomas, melanomas, carcinomas, and gliomas by inhibiting tumor angiogenesis.

Thus, it is contemplated that a wide variety of cancers may be treated using the peptides of the present invention including cancers of the brain (glioblastoma, astrocytoma, oligodendroglioma, ependymomas), lung, liver, spleen, kidney, lymph node, pancreas, small intestine, blood cells, colon, stomach, breast, endometrium, prostate, testicle, ovary, skin, head and neck, esophagus, bone marrow, blood or other tissue.

In many contexts, it is not necessary that the tumor cell be killed or induced to undergo normal cell death or "apoptosis." Rather, to accomplish a meaningful treatment, all that is required is that the tumor growth be slowed to some degree or localized to a specific area and inhibited from spread to disparate sites. It may be that the tumor growth is completely blocked, however, or that some tumor regression is achieved. Clinical terminology such as "remission" and "reduction of tumor" burden also are contemplated given their normal usage. In the context of the present invention, the therapeutic effect may result from an inhibition of angiogenesis and/or an inhibition of lymphangiogenesis.

VEGF-C and VEGF-D of the VEGF family of growth factors have utility for preventing stenosis or restenosis of blood vessels. See International Patent Application No. PCT/US99/24054 (WO 00/24412), "Use of VEGF-C or VEGF-D Gene or Protein to Prevent Restenosis," filed October 26, 1999, incorporated herein by reference in its entirety. The polypeptides and polynucleotides of the invention also will have utility for these indications and can substitute for VEGF-C and VEGF-D with respect to the materials and methods described therein. Thus, in another aspect, the invention provides a method of treating a mammalian subject to prevent stenosis or restenosis of a blood vessel, comprising the step of administering to a mammalian subject in need of treatment to prevent stenosis or restenosis of a blood

vessel a composition comprising one or more polypeptide(s) or polynucleotide(s) of the invention, in an amount effective to prevent stenosis or restenosis of the blood vessel. In a preferred embodiment, the administering comprises implanting an intravascular stent in the mammalian subject, where the stent is coated or impregnated with the composition. Exemplary materials for constructing a drug-coated or drug-impregnated stent are described in literature cited above and reviewed in Lincoff *et al., Circulation*, 90: 2070-2084 (1994). In another preferred embodiment, the composition comprises microparticles composed of biodegradable polymers such as PGLA, non-degradable polymers, or biological polymers (e.g., starch) which particles encapsulate or are impregnated by a polypeptide(s) of the invention. Such particles are delivered to the intravascular wall using, e.g., an infusion angioplasty catheter. Other techniques for achieving locally sustained drug delivery are reviewed in Wilensky *et al., Trends Caridovasc. Med.*, 3:163-170 (1993), incorporated herein by reference.

Administration via one or more intravenous injections subsequent to the angioplasty or bypass procedure also is contemplated. Localization of the polypeptides of the invention to the site of the procedure occurs due to expression of VEGF receptors on proliferating endothelial cells and due to heparin sulfate binding property of the molecules of the present invention. Localization is further facilitated by recombinantly expressing the polypeptides of the invention as a fusion polypeptide (e.g., fused to an apolipoprotein B-100 oligopeptide as described in Shih *et al., Proc. Nat'l. Acad. Sci. USA*, 87:1436-1440 (1990). Co-administration of polynucleotides and polypeptides of the invention is also contemplated.

Likewise, the invention also provides surgical devices that are used to treat circulatory disorders, such as intravascular or endovascular stents, balloon catheters, infusion-perfusion catheters, extravascular collars, elastomeric membranes, and the like, which have been improved by coating with, impregnating with, adhering to, or encapsulating within the device a composition comprising a polynucleotide or polypeptide of the invention.

Polynucleotides or polypeptides of the invention can be administered purely as a prophylactic treatment to prevent stenosis, or shortly before, and/or concurrently with, and/or shortly after a percutaneous transluminal coronary angioplasty procedure, for the purpose of preventing restenosis of the subject vessel.

In another preferred embodiment, the polynucleotide or polypeptide is administered before, during, and/or shortly after a bypass procedure (e.g., a coronary bypass procedure), to prevent stenosis or restenosis in or near the transplanted (grafted) vessel, especially stenosis at the location of the graft itself. In yet another embodiment, the polynucleotide or polypeptide is administered before, during, or after a vascular transplantation in the vascular periphery that has been performed to treat peripheral ischemia or intermittent claudication. By prevention of stenosis or restenosis is meant prophylactic treatment to reduce the amount/severity of, and/or substantially eliminate, the stenosis or restenosis that frequently occurs in such surgical procedures. The polynucleotide or polypeptide is included in the composition in an amount and in a form effective to promote stimulation of VEGF receptors in a blood vessel of the mammalian subject, thereby preventing stenosis or restenosis of the blood vessel.

In a preferred embodiment, the mammalian subject is a human subject. For example, the subject is a person suffering from coronary artery disease that has been identified by a cardiologist as a candidate who could benefit from a therapeutic balloon angioplasty (with or without insertion of an intravascular stent) procedure or from a coronary bypass procedure. Practice of methods of the invention in other mammalian subjects, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g., primate, porcine, canine, or rabbit animals), also is contemplated.

The polypeptides of the invention may be used to modulate the growth of isolated cells or cell lines. For example, certain neoplastic disease states are characterized by the appearance of VEGF receptors on cell surfaces [Valtola *et al., Am J Path 154*:1381-90 (1999)]. Polypeptides of the invention may be screened to determine the ability of the polypeptide to modulate the growth of the neoplastic cells. Other disease states are likely characterized by mutations in VEGF receptors [Ferrell *et al., Hum Mol Genetics 7*:2073-78 (1998)]. Polypeptides of the invention that modulate the activity of the mutant forms of the VEGF receptor in a manner different than naturally-occurring vascular endothelial growth factors will be useful at modulating the symptoms and severity of such disease states.

Polypeptides of the invention may be used to modulate the growth of stem cells, progenitor cells for various tissues, and primary cell isolates that express receptor for the polypeptides.

As indicated herein above, and discussed further in U.S. Patent Application No. 10/669,176, filed September 23, 2003, VEGF-C compositions are useful in the treatment of neurological disorders. The compositions of the invention are useful in the treatment of such disorders either alone or in conjunction with additional therapeutics, such as a neural growth factor. Exemplary neural growth factors include, but are not limited to, interferon gamma, nerve growth factor, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), neurogenin, brain derived neurotrophic factor (BDNF), thyroid hormone, bone morphogenic proteins (BMPs), leukemia inhibitory factor (LIF), sonic hedgehog, and glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), interleukins, interferons, stem cell factor (SCF), activins, inhibins, chemokines, retinoic acid and ciliary neurotrophic factor (CNTF). In one aspect, the invention contemplates a composition comprising a heparin binding VEGFR-3 ligand of the invention and a neural growth factor in a pharmaceutically acceptable diluent or carrier, or polynucleotides comprising the same.

Methods of the invention preferably are performed wherein the subject has a disease or condition characterized by aberrant growth of neuronal cells, neuronal scarring and damage or neural degeneration. A disease or medical disorder is considered to be nerve damage if the survival or function of nerve cells and/or their axonal processes is compromised. Such nerve damage occurs as the result of conditions including: physical injury, which causes the degeneration of the axonal processes and/or nerve cell bodies near the site of the injury; ischemia, as a stroke; exposure to neurotoxins, such as the cancer and AIDS chemotherapeutic agents such as cisplatin and dideoxycytidine (ddC), respectively; chronic metabolic diseases, such as diabetes or renal dysfunction; and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Amyotrophic Lateral Sclerosis (ALS), which cause the degeneration of specific neuronal populations. Conditions involving nerve damage include Parkinson's disease, Alzheimer's disease, Amyotrophic Lateral Sclerosis, stroke, diabetic polyneuropathy, toxic neuropathy, glial scar, and physical damage to the nervous system such as that caused by physical injury of the brain and

spinal cord or crush or cut injuries to the arm and hand or other parts of the body, including temporary or permanent cessation of blood flow to parts of the nervous system, as in stroke.

In one embodiment, the disease or condition being treated is a neurodegenerative disorder, wherein the neurodegenerative disorder is selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease, Amyotrophic Lateral Sclerosis (ALS), dementia and cerebral palsy. In another embodiment, the disease or condition is selected from the group consisting of neural trauma or neural injury. Methods of the invention also can be performed to treat or ameliorate the effects of neural trauma or injury, such as injury related to stroke, spinal cord injury, post-operative injury, brain ischemia and other traumas.

The invention can be used to treat one or more adverse consequences of central nervous system injury that arise from a variety of conditions. Thrombus, embolus, and systemic hypotension are among the most common causes of stroke. Other injuries may be caused by hypertension, hypertensive cerebral vascular disease, rupture of an aneurysm, an angioma, blood dyscrasia, cardiac failure, cardiac arrest, cardiogenic shock, kidney failure, septic shock, head trauma, spinal cord trauma, seizure, bleeding from a tumor, or other loss of blood volume or pressure. These injuries lead to disruption of physiologic function, subsequent death of neurons, and necrosis (infarction) of the affected areas. The term "stroke" connotes the resulting sudden and dramatic neurologic deficits associated with any of the foregoing injuries.

The terms "ischemia" or "ischemic episode," as used herein, means any circumstance that results in a deficient supply of blood to a tissue. Thus, a central nervous system ischemic episode results from an insufficiency or interruption in the blood supply to any locus of the brain such as, but not limited to, a locus of the cerebrum, cerebellum or brain stem. The spinal cord, which is also a part of the central nervous system, is equally susceptible to ischemia resulting from diminished blood flow. An ischemic episode may be caused by a constriction or obstruction of a blood vessel, as occurs in the case of a thrombus or embolus. Alternatively, the ischemic episode may result from any form of compromised cardiac function, including cardiac arrest, as described above. Where the deficiency is sufficiently severe and prolonged, it can lead to disruption of physiologic function, subsequent

death of neurons, and necrosis (infarction) of the affected areas. The extent and type of neurologic abnormality resulting from the injury depend on the location and size of the infarct or the focus of ischemia. Where the ischemia is associated with a stroke, it can be either global or focal in extent.

Polypeptides and polynucleotide compositions of the invention will also be useful for treating traumatic injuries to the central nervous system that are caused by mechanical forces, such as a blow to the head. Trauma can involve a tissue insult selected from abrasion, incision, contusion, puncture, compression, etc., such as can arise from traumatic contact of a foreign object with any locus of or appurtenant to the mammalian head, neck or vertebral column. Other forms of traumatic injury can arise from constriction or compression of mammalian CNS tissue by an inappropriate accumulation of fluid (e.g., a blockade or dysfunction of normal cerebrospinal fluid or vitreous humour fluid production, turnover or volume regulation, or a subdural or intracranial hematoma or edema). Similarly, traumatic constriction or compression can arise from the presence of a mass of abnormal tissue, such as a metastatic or primary tumor.

It is further contemplated that methods of the invention directed to neurological indications can be practiced by co-administering a polypeptide of the formula X-B-Z or Z-B-X with a neurotherapeutic agent. By "neurotherapeutic agent" is meant an agent used in the treatment of neurodegenerative diseases or to treat neural trauma and neural injury. Exemplary neurotherapeutic agents include tacrine (Cognex), donepezil (Aricept), rivastigmine (Exelon), galantamine (Reminyl), and cholinesterase inhibitors and anti-inflammatory drugs, which are useful in the treatment of Alzheimer's disease as well as other neurodegenerative diseases.

Additional neurotherapeutic agents include anti-cholinergics, dopamine agonists, catechol-0-methyl-transterases (COMTs), amantadine (Symmetrel), Sinemet®, Selegiline, carbidopa, ropinirole (Requip), coenzyme Q10, Pramipexole (Mirapex) and levodopa (L-dopa), which are useful in the treatment of Parkinson's disease as well as other neurodegenerative diseases. Other therapeutics agents for the treatment of neurological disorders will be known to those of skill in the art and may be useful in the combination therapies contemplated herein.

E. Pharmaceutical Compositions Comprising Chimeric VEGF polypeptides

Polypeptides and/or polynucleotides of the invention may be administered in any suitable manner using an appropriate pharmaceutically-acceptable vehicle, *e.g.*, a pharmaceutically-acceptable diluent, adjuvant, excipient or carrier. The composition to be administered according to methods of the invention preferably comprises (in addition to the polynucleotide or vector) a pharmaceutically-acceptable carrier solution such as water, saline, phosphate-buffered saline, glucose, or other carriers conventionally used to deliver therapeutics intravascularly. Multi-gene therapy is also contemplated, in which case the composition optionally comprises both the polynucleotide of the invention/vector and another polynucleotide/vector selected to prevent restenosis or other disorder mediated through the action of a VEGF receptor. Exemplary candidate genes/vectors for co-transfection with transgenes encoding polypeptides of the invention are described in the literature cited above, including genes encoding cytotoxic factors, cytostatic factors, endothelial growth factors, and smooth muscle cell growth/migration inhibitors.

The "administering" that is performed according to the present method may be performed using any medically-accepted means for introducing a therapeutic directly or indirectly into the vasculature of a mammalian subject, including but not limited to injections (*e.g.*, intravenous, intramuscular, subcutaneous, or catheter); oral ingestion; intranasal or topical administration; and the like. In a preferred embodiment, administration of the composition comprising a polynucleotide of the invention is performed intravascularly, such as by intravenous, intra-arterial, or intracoronary arterial injection. The therapeutic composition may be delivered to the patient at multiple sites. The multiple administrations may be rendered simultaneously or may be administered over a period of several hours. In certain cases it may be beneficial to provide a continuous flow of the therapeutic composition. Additional therapy may be administered on a period basis, for example, daily, weekly or monthly. To minimize angiogenic side effects in non-target tissues, preferred methods of administration are methods of local administration, such as admistration by intramuscular injection.

In general, peroral dosage forms for the therapeutic delivery of polypeptides is ineffective because in order for such a formulation to the efficacious, the peptide must be protected from the enzymatic environment of the gastrointestinal

tract. Additionally, the polypeptide must be formulated such that it is readily absorbed by the epithelial cell barrier in sufficient concentrations to effect a therapeutic outcome. The chimeric polypeptides of the present invention may be formulated with uptake or absorption enhancers to increase their efficacy. Such enhancer include for example, salicylate, glycocholate/linoleate, glycholate, aprotinin, bacitracin, SDS caprate and the like. An additional detailed discussion of oral formulations of peptides for therapeutic delivery is found in Fix, *J. Pharm. Sci.*, 85(12) 1282-1285, 1996, and Oliyai and Stella, *Ann. Rev. Pharmacol. Toxicol.*, 32:521-544, 1993, both incorporated by reference.

The amounts of peptides in a given dosage will vary according to the size of the individual to whom the therapy is being administered as well as the characteristics of the disorder being treated. In exemplary treatments, it may be necessary to administer about 50mg/day, 75 mg/day, 100mg/day, 150mg/day, 200mg/day, 250 mg/day. These concentrations may be administered as a single dosage form or as multiple doses.

The polypeptides may also be employed in accordance with the present invention by expression of such polypeptide *in vivo*, which is often referred to as gene therapy. The present invention provides a recombinant DNA vector containing a heterologous segment encoding a chimeric polypeptide of the invention that is capable of being inserted into a microorganism or eukaryotic cell and that is capable of expressing the encoded chimeric protein.

In a highly preferred embodiment, the composition is administered locally. Thus, in the context of treating restenosis or stenosis, administration directly to the site of angioplasty or bypass is preferred. For example, the administering comprises a catheter-mediated transfer of the transgene-containing composition into a blood vessel of the mammalian subject, especially into a coronary artery of the mammalian subject. Exemplary materials and methods for local delivery are reviewed in Lincoff *et al., Circulation*, 90: 2070-2084 (1994); and Wilensky *et al., Trends Cardiovasc. Med.*, 3:163-170 (1993), both incorporated herein by reference. For example, the composition is administered using infusion-perfusion balloon catheters (preferably microporous balloon catheters) such as those that have been described in the literature for intracoronary drug infusions. See, e.g., U.S. Patent No. 5,713,860 (Intravascular Catheter with Infusion Array); U.S. Patent No. 5,087,244;

U.S. Patent No. 5,653,689; and Wolinsky *et al., J. Am. Coll. Cardiol.*, 15: 475-481 (1990) (Wolinsky Infusion Catheter); and Lambert *et al., Coron. Artery Dis.*, 4: 469-475 (1993), all of which are incorporated herein by reference in their entirety. Use of such catheters for site-directed somatic cell gene therapy is described, e.g., in Mazur *et al., Texas Heart Institute Journal*, 21; 104-111 (1994), incorporated herein by reference. In an embodiment where the transgene encoding a chimeric polypeptide of the invention is administered in an adenovirus vector, the vector is preferably administered in a pharmaceutically acceptable carrier at a dose of 10^7-10^{13} viral particles, and more preferably at a dose of 10^9-10^{11} viral particles. The adenoviral vector composition preferably is infused over a period of 15 seconds to 30 minutes, more preferably 1 to 10 minutes.

For example, in patients with angina pectoris due to a single or multiple lesions in coronary arteries and for whom PTCA is prescribed on the basis of primary coronary angiogram findings, an exemplary protocol involves performing PTCA through a 7F guiding catheter according to standard clinical practice using the femoral approach. If an optimal result is not achieved with PTCA alone, then an endovascular stent also is implanted. (A nonoptimal result is defined as residual stenosis of > 30 % of the luminal diameter according to a visual estimate, and B or C type dissection.) Arterial gene transfer at the site of balloon dilatation is performed with a replication-deficient adenoviral vector expressing a polypeptide of the invention immediately after the angioplasty, but before stent implantation, using an infusion-perfusion balloon catheter. The size of the catheter will be selected to match the diameter of the artery as measured from the angiogram, varying, e.g., from 3.0 to 3.5F in diameter. The balloon is inflated to the optimal pressure and gene transfer is performed during a 10 minute infusion at the rate of 0.5 ml/min with virus titer of 1.15×10^{10} pfu/ml.

In another embodiment, intravascular administration with a gel-coated catheter is contemplated, as has been described in the literature to introduce other transgenes. See, e.g., U.S. Patent No. 5,674,192 (Catheter coated with tenaciously-adhered swellable hydrogel polymer); Riessen *et al., Human Gene Therapy*, 4: 749-758 (1993); and Steg *et al., Circulation*, 96: 408-411 (1997) and 90: 1648-1656 (1994); all incorporated herein by reference. Briefly, DNA in solution (e.g., a polynucleotide of the invention) is applied one or more times *ex vivo* to the

surface of an inflated angioplasty catheter balloon coated with a hydrogel polymer (e.g., Slider with Hydroplus, Mansfield Boston Scientific Corp., Watertown, MA). The Hydroplus coating is a hydrophilic polyacrylic acid polymer that is cross-linked to the balloon to form a high molecular weight hydrogel tightly adhered to the balloon. The DNA covered hydrogel is permitted to dry before deflating the balloon. Re-inflation of the balloon intravascularly, during an angioplasty procedure, causes the transfer of the DNA to the vessel wall.

In yet another embodiment, an expandable elastic membrane or similar structure mounted to or integral with a balloon angioplasty catheter or stent is employed to deliver the transgene encoding a polypeptide of the invention. See, e.g., U.S. Patent Nos. 5,707,385, 5,697,967, 5,700,286, 5,800,507, and 5,776,184, all incorporated by reference herein.

In another variation, the composition containing the transgene encoding a polypeptide of the invention is administered extravascularly, e.g., using a device to surround or encapsulate a portion of vessel. See, e.g., International Patent Publication WO 98/20027, incorporated herein by reference, describing a collar that is placed around the outside of an artery (e.g., during a bypass procedure) to deliver a transgene to the arterial wall via a plasmid or liposome vector.

In still another variation, endothelial cells or endothelial progenitor cells are transfected *ex vivo* with the transgene encoding a polypeptide of the invention, and the transfected cells as administered to the mammalian subject. Exemplary procedures for seeding a vascular graft with genetically modified endothelial cells are described in U.S. Patent No. 5,785,965, incorporated herein by reference.

In preferred embodiments, polynucleotides of the invention further comprises additional sequences to facilitate the gene therapy. In one embodiment, a "naked" transgene encoding a polypeptide of the invention (i.e., a transgene without a viral, liposomal, or other vector to facilitate transfection) is employed for gene therapy. In this embodiment, the polynucleotide of the invention preferably comprises a suitable promoter and/or enhancer sequence (e.g., cytomegalovirus promoter/enhancer [Lehner *et al., J. Clin. Microbiol.*, 29:2494-2502 (1991); Boshart *et al., Cell*, 41:521-530 (1985)]; Rous sarcoma virus promoter [Davis *et al., Hum.*

Gene Ther., 4:151 (1993)]; Tie promoter [Korhonen *et al.*, *Blood*, 86(5): 1828-1835 (1995)]; or simian virus 40 promoter) for expression in the target mammalian cells, the promoter being operatively linked upstream (i.e., 5') of the polypeptide-coding sequence. The polynucleotides of the invention also preferably further includes a suitable polyadenylation sequence (e.g., the SV40 or human growth hormone gene polyadenylation sequence) operably linked downstream (i.e., 3') of the polypeptide-coding sequence. The polynucleotides of the invention also preferably comprise a nucleotide sequence encoding a secretory signal peptide fused in-frame with the polypeptide sequence. The secretory signal peptide directs secretion of the polypeptide of the invention by the cells that express the polynucleotide, and is cleaved by the cell from the secreted polypeptide. The signal peptide sequence can be that of another secreted protein, or can be a completely synthetic signal sequence effective to direct secretion in cells of the mammalian subject.

The polynucleotide may further optionally comprise sequences whose only intended function is to facilitate large-scale production of the vector, e.g., in bacteria, such as a bacterial origin of replication and a sequence encoding a selectable marker. However, in a preferred embodiment, such extraneous sequences are at least partially cleaved off prior to administration to humans according to methods of the invention. One can manufacture and administer such polynucleotides for gene therapy using procedures that have been described in the literature for other transgenes. See, e.g., Isner *et al.*, *Circulation*, 91: 2687-2692 (1995); and Isner *et al.*, *Human Gene Therapy*, 7: 989-1011 (1996); incorporated herein by reference in the entirety.

Any suitable vector may be used to introduce the transgene encoding one of the polypeptides of the invention, into the host. Exemplary vectors that have been described in the literature include replication-deficient retroviral vectors, including but not limited to lentivirus vectors [Kim *et al.*, *J. Virol.*, 72(1): 811-816 (1998); Kingsman & Johnson, Scrip Magazine, October, 1998, pp. 43-46.]; adeno-associated viral vectors [U.S. Patent No. 5,474,935; U.S. Patent No. 5,139,941; U.S. Patent No. 5,622,856; U.S. Patent No. 5,658,776; U.S. Patent No. 5,773,289; U.S. Patent No. 5,789,390; U.S. Patent No. 5,834,441; U.S. Patent No. 5,863,541; U.S. Patent No. 5,851,521; U.S. Patent No. 5,252,479; Gnatenko *et al.*, *J. Investig. Med.*, 45: 87-98 (1997)]; adenoviral vectors [See, e.g., U.S. Patent No.

5,792,453; U.S. Patent No. 5,824,544; U.S. Patent No. 5,707,618; U.S. Patent No. 5,693,509; U.S. Patent No. 5,670,488; U.S. Patent No. 5,585,362; Quantin *et al.*, *Proc. Natl. Acad. Sci. USA*, 89: 2581-2584 (1992); Stratford-Perricadet *et al.*, *J. Clin. Invest.*, 90: 626-630 (1992); and Rosenfeld *et al.*, *Cell,* 68: 143-155 (1992)]; an adenoviral-adenoassociated viral chimeric (see for example, U.S. Patent No. 5,856,152) or a vaccinia viral or a herpesviral (see for example, U.S. Patent No. 5,879,934; U.S. Patent No. 5,849,571; U.S. Patent No. 5,830,727; U.S. Patent No. 5,661,033; U.S. Patent No. 5,328,688; Lipofectin-mediated gene transfer (BRL); liposomal vectors [See, e.g., U.S. Patent No. 5,631,237 (Liposomes comprising Sendai virus proteins)] ; and combinations thereof. All of the foregoing documents are incorporated herein by reference in their entirety. Replication-deficient adenoviral vectors constitute a preferred embodiment.

Other non-viral delivery mechanisms contemplated include calcium phosphate precipitation (Graham and Van Der Eb, *Virology*, 52:456-467, 1973; Chen and Okayama, *Mol. Cell Biol.*, 7:2745-2752, 1987; Rippe *et al.*, *Mol. Cell Biol.*, 10:689-695, 1990) DEAE-dextran (Gopal, *Mol. Cell Biol.*, 5:1188-1190, 1985), electroporation (Tur-Kaspa *et al.*, *Mol. Cell Biol.*, 6:716-718, 1986; Potter *et al.*, *Proc. Nat. Acad. Sci. USA*, 81:7161-7165, 1984), direct microinjection (Harland and Weintraub, *J. Cell Biol.*, 101:1094-1099, 1985.), DNA-loaded liposomes (Nicolau and Sene, *Biochim. Biophys. Acta*, 721:185-190, 1982; Fraley *et al.*, *Proc. Natl. Acad. Sci. USA*, 76:3348-3352, 1979; Felgner, *Sci Am.* 276(6):102-6, 1997; Felgner, *Hum Gene Ther.* 7(15):1791-3, 1996), cell sonication (Fechheimer *et al.*, *Proc. Natl. Acad. Sci. USA,* 84:8463-8467, 1987), gene bombardment using high velocity microprojectiles (Yang *et al.*, *Proc. Natl. Acad. Sci USA,* 87:9568-9572, 1990), and receptor-mediated transfection (Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987; Wu and Wu, *Biochemistry*, 27:887-892, 1988; Wu and Wu, *Adv. Drug Delivery Rev.*, 12:159-167, 1993).

The expression construct (or indeed the polypeptides discussed above) may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed

structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, *In: Liver diseases, targeted diagnosis and therapy using specific receptors and ligands,* Wu G, Wu C ed., New York: Marcel Dekker, pp. 87-104, 1991). The addition of DNA to cationic liposomes causes a topological transition from liposomes to optically birefringent liquid-crystalline condensed globules (Radler *et al.*, *Science*, 275(5301):810-4, 1997). These DNA-lipid complexes are potential non-viral vectors for use in gene therapy and delivery.

Liposome-mediated nucleic acid delivery and expression of foreign DNA *in vitro* has been successful. Also contemplated in the present invention are various commercial approaches involving "lipofection" technology. In certain embodiments of the invention, the liposome may be complexed with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (Kaneda *et al.*, *Science*, 243:375-378, 1989). In other embodiments, the liposome may be complexed or employed in conjunction with nuclear nonhistone chromosomal proteins (HMG-1) (Kato *et al.*, *J. Biol. Chem.*, 266:3361-3364, 1991). In yet further embodiments, the liposome may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression constructs have been successfully employed in transfer and expression of nucleic acid *in vitro* and *in vivo*, then they are applicable for the present invention.

Other vector delivery systems that can be employed to deliver a nucleic acid encoding a therapeutic gene into cells include receptor-mediated delivery vehicles. These take advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost all eukaryotic cells. Because of the cell type-specific distribution of various receptors, the delivery can be highly specific (Wu and Wu, 1993, *supra*).

In other embodiments, the delivery vehicle may comprise a ligand and a liposome. For example, Nicolau *et al.* (*Methods Enzymol.*, 149:157-176, 1987) employed lactosyl-ceramide, a galactose-terminal asialganglioside, incorporated into liposomes and observed an increase in the uptake of the insulin gene by hepatocytes. Thus, it is feasible that a nucleic acid encoding a therapeutic gene also may be specifically delivered into a particular cell type by any number of receptor-ligand systems with or without liposomes.

In another embodiment of the invention, the expression construct may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above that physically or chemically permeabilize the cell membrane. This is applicable particularly for transfer *in vitro*, however, it may be applied for *in vivo* use as well. Dubensky *et al.* (*Proc. Nat. Acad. Sci. USA*, 81:7529-7533, 1984) successfully injected polyomavirus DNA in the form of $CaPO_4$ precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Neshif (*Proc. Nat. Acad. Sci. USA*, 83:9551-9555, 1986) also demonstrated that direct intraperitoneal injection of $CaPO_4$ precipitated plasmids results in expression of the transfected genes.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, *Nature*, 327:70-73, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, *Proc. Natl. Acad. Sci USA*, 87:9568-9572, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

In embodiments employing a viral vector, preferred polynucleotides still include a suitable promoter and polyadenylation sequence as described above. Moreover, it will be readily apparent that, in these embodiments, the polynucleotide further includes vector polynucleotide sequences (e.g., adenoviral polynucleotide sequences) operably connected to the sequence encoding a polypeptide of the invention.

Thus, in one embodiment the composition to be administered comprises a vector, wherein the vector comprises a polynucleotide of the invention. In a preferred embodiment, the vector is an adenovirus vector. In a highly preferred embodiment, the adenovirus vector is replication-deficient, i.e., it cannot replicate in the mammalian subject due to deletion of essential viral-replication sequences from the adenoviral genome. For example, the inventors contemplate a method wherein the vector comprises a replication-deficient adenovirus, the adenovirus comprising the

polynucleotide of the invention operably connected to a promoter and flanked on either end by adenoviral polynucleotide sequences.

Similarly, the invention includes kits which comprise compounds or compositions of the invention packaged in a manner which facilitates their use to practice methods of the invention. In a simplest embodiment, such a kit includes a compound or composition described herein as useful for practice of the invention (e.g., polynucleotides or polypeptides of the invention), packaged in a container such as a sealed bottle or vessel, with a label affixed to the container or included in the package that describes use of the compound or composition to practice the method of the invention. Preferably, the compound or composition is packaged in a unit dosage form. In another embodiment, a kit of the invention includes a composition of both a polynucleotide or polypeptide packaged together with a physical device useful for implementing methods of the invention, such as a stent, a catheter, an extravascular collar, a polymer film, or the like. In another embodiment, a kit of the invention includes compositions of both a polynucleotide or polypeptide of the invention packaged together with a hydrogel polymer, or microparticle polymers, or other carriers described herein as useful for delivery of the polynucleotides or polypeptides to the patient.

Example 1
Recombinant VEGF-C with heparin binding property

The present Example describes the generation of chimeric VEGF-C molecules comprising an amino terminal VEGFR-3 binding domain of VEGF-C fused to a carboxy terminal heparin binding domain from VEGF. These molecules retain VEGFR-3 binding activity as shown by a cell survival assay and are expected to have an enhanced heparin binding activity as compared to native VEGF-C and enhanced angiogenic and/or lymphangiogenic properties.

Materials & Methods

Cloning: cDNAs encoding the fusion proteins comprised of the VEGF homology domain of VEGF-C and the C-terminus of VEGF (exon 6-8 encoded polypeptide fragment, referred to below as CA89, or exon 6-7 encoded fragment referred to below as CA65) were constructed by PCR amplification using the

following primers: VEGF-CΔNΔC, 5′-ACATTGGTGTGCACCTCCAAGC - 3 ′ (SEQ ID NO: 16) and 5 ′ -AATAATGGAATGAACTTGTCTGTAAAC-3′ (SEQ ID NO: 17); VEGF C-terminal regions: 5′-AAATCAGTTCGAGGAAAGGGAAAG-3′ (SEQ ID NO: 18) or 5′-CCCTGTGGGCCTTGCTCAGAG-3′ (SEQ ID NO: 19), and 5′-CCATGCTCGAGAGTCTTTCCTGGTGAGAGATCTGG-3′ (SEQ ID NO: 20). The PCR products were digested with HindIII (5′-HindIII/3′-blunt) or XhoI (5′-blunt-3′-XhoI), and cloned into the pEBS7 (Peterson and Legerski, *Gene*, 107 279-84, 1991)) expression vector that had been digested with the same enzymes to create clones named pEBS7/CA89 and pEBS7/CA65. The inserts were also subcloned into pREP7 at HindIII/XhoI sites (pREP7/CA89 and pREP7/CA65.

Cell culture, transfection and immunoprecipitation: 293T and 293EBNA cells were maintained in DMEM medium supplemented with 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum (Autogen Bioclear). BaF3 cells (Achen *et al., Eur J Biochem.*, 267: 2505-15, 2000) were grown in DMEM as above with the addition of Zeocine (200 μg/ml) and the recombinant human VEGF-CΔNΔC (100 ng/ml).

293T cells were transfected with pEBS7/CA89, pEBS7/CA65 or the pEBS7 vector using liposomes (FuGENE 6, Roche). Cells transfected with pEBS7/CA89 were cultured with or without heparin (20 unit/ml). Transfected cells were cultured for 24 h, and were then metabolically labeled in methionine-free and cysteine-free modified Eagle medium supplemented with [^{35}S]methionine/[^{35}S]cysteine (Promix, Amersham Pharmacia Biotech) at 100 μCi/mL for 8 h. Conditioned medium was then harvested, cleared of particulate material by centrifugation, and incubated with polyclonal antibodies against VEGF-C [Joukov *et al., EMBO J.* 16:3898-911, 1997). The formed antigen-antibody complexes were bound to protein A Sepharose (Pharmacia Biotech), which were then washed twice with 0.5% bovine serum albumin/0.02% Tween 20 in phosphate-buffered saline (PBS) and once with PBS, and analysed in sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE) under reducing conditions.

293EBNA cells were transfected with pREP7/CA89, pREP7/CA65 or the pREP7 vector as described above. Cells transfected with pREP7/CA89 were cultured with or without heparin (20 unit/ml). The transfected cells were cultured for two days, and the supernatants were harvested for the assay of biological activity.

Bioassay for growth factor-mediated cell survival: Ba/F3 cells expressing a VEGFR-3/EpoR chimeric receptor (Achen *et al., Eur J Biochem.*, 267: 2505-15, 2000) were seeded in 96-well plates at 15,000 cells/well in triplicates supplied with conditioned medium (0, 1, 5, 10 or 20 µl) from cell cultures transfected with pREP7/CA89, pREP7/CA65 or the pREP7 vector. Cell viability was measured by a colorimetric assay. MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma), 0.5 mg/ml) was added into each well and incubated for 4 h at 37°C. The reaction was terminated by adding 100 µl of lysis buffer (10% SDS, 10 mM HCl), and the resulting formazan products were solubilized overnight at 37°C in a humid atmosphere. The absorbance at 540 nm was measured with a Multiscan microtiter plate reader (Labsystems).

Results & Discussion

The innate heparin binding property of certain growth factors has been implicated as important for their biological activities (Dougher *et al., Growth Factors,* 14: 257-68, 1997; Carmeliet *et al., Nat Med* 5: 495-502, 1999; Ruhrberg *et al., Genes Dev* 16 2684-98, 2002). VEGF (Poltorak *et al., J. Biol. Chem.* 272:7151-8, 1997; Gitay-Goren *et al., J Biol Chem* 271: 5519-23, 1996), VEGF-B_{167} (Makinen *et al., J. Biol. Chem.*, 274:21217-22, 1999), and PlGF-2 (Hauser and Weich, *Growth Factors,* 9 259-68, 1993) all possess significant heparin binding activity, but VEGF-C and VEGF-D do not. Both of these latter molecules have been shown to induce lymphangiogenesis in transgenic mice and in other *in vivo* models (Jeltsch *et al., Science* 276:1423-5, 1997; Oh *et al., Dev Biol* 188: 96-109, 1997; Veikkola *et al., EMBO J* 20: 1223-31, 2001). Although recombinant proteins of mature forms of VEGF-C and VEGF-D are believed to exert angiogenic activity via VEGFR-2 (Cao *et al. Proc Natl Acad Sci U S A* 95: 14389-94, 1998; Marconcini *et al., Proc Natl Acad Sci U S A* 96: 9671-6, 1999), mature forms of VEGF-C delivered by other means such as adenoviral vectors have so far induced weak lymphangiogenic activity and little, if any, angiogenic activity in mice. These data suggest that the concentration of the protein present may not be sufficient, or that the half-life of the mature form of VEGF-C protein may be too short to induce a potent angiogenic effect. Maximum activation of VEGFR-2 *in vivo* may also require the ligand to have the property of

heparin binding, as suggested for VEGF (Dougher *et al., Growth Factors,* 14: 257-68, 1997).

To investigate the effects of introducing a heparin binding activity on the angiogenic and lymphangiogenic effects of VEGF-C, plasmids encoding chimeric proteins comprised of the signal sequence and the VEGF homology domain (VHD) of VEGF-C, and VEGF exon 6-8 or exon 7-8 encoded sequences (Fig. 1C) were constructed. Expression of the chimeric VEGF-C proteins by the transfected cells was confirmed by immunoprecipitation with polyclonal antibodies against VEGF-C (Fig. 1D). CA65 was secreted and released into the supernatant, but CA89 was not released into the supernatant unless heparin was included in the culture medium (Fig. 1D), indicating that it apparently binds to cell surface heparan sulfates similar to what has been described for $VEGF_{189}$. VEGFR-3-mediated biological activity of the chimeric proteins (CA89 and CA65) was demonstrated by a bioassay using Ba/F3 cells expressing a chimeric VEGFR-3/erythropoietin (Epo) receptor (Ba/F3/VEGFR-3). Conditioned medium from both 293EBNA/CA89 and 293EBNA/CA65 cells were shown to induce survival and proliferation of the IL-3 dependent Ba/F3/VEGFR-3 cells in the absence of the recombinant IL-3 protein (Fig. 2). The effect was detectable even with 1 µl of the conditioned medium added.

Lymphatic vessels typically accompany blood vessels. The chimeric molecules of the present invention may allow efficient localization of growth factors expressed in a given tissue, without the danger of obtaining aberrant side effects in other sites/organs. Secondly, the heparin binding forms would allow a growth factor gradient to be established for vessel sprouting. Further, given the teachings described herein, the chimeric polypeptides of the present invention which are heparin binding factors give enhanced lymphangiogenic and/or angiogenic effects, as their three dimensional diffusion is replaced by two-dimensional diffusion in the plane of the cell surface heparin matrix, which leads to a more concentrated form of the growth factor available for the high-affinity signal transducing receptors. Furthermore, heparin binding forms of VEGF containing the VEGF exon 7-encoded sequence can also bind to neuropilins, which have important roles in the development of the cardiovascular system and the lymphatic system. Consequently, the putative neuropilin-1 binding property of the chimeric polypeptides of the invention could direct VEGF-C towards more efficient stimulation of angiogenesis.

Example 2

VEGF-C fused to heparin-binding domain has increased lymphangiogenic activity

The present example further demonstrates that chimeric VEGF-C molecules containing a heparin binding domain have increased lymphangiogenic activity in comparison with the VEGF-CΔNΔC form. The enhancement of the biological activity may result from an increased bioavailability of the protein, or increased receptor binding via binding to NP-1 or NP-2. Without being bound to any theory of mechanism of action, it is possible that the presence of the heparin binding domain facilitates a two-dimensional diffusion of the heparin-domain-containing chimeric VEGF-C molecules such that the chimeric molecules become distributed in the plane of the cell surface heparan sulphate matrix, which leads to a more concentrated form of the growth factor presented and available for the high-affinity signal-transducing receptors. Furthermore, the heparin binding forms may allow a growth factor gradient to be established for vessel sprouting.

Materials and Methods

The methods described in Example 1 are incorporated into the present Example by reference. The studies described in the present example also employed the following additional experimental protocols.

Production and in vivo delivery of CA89 and CA65 by viral vectors. The AAV vector psub-CAG-WPRE was cloned by substituting the CMV promoter fragment of psub-CMV-WPRE (Paterna *et al., Gene Ther.*, 7(15):1304-1311, 2000) with the CMV-chicken beta-actin insert (Niwa *et al., Gene*, 108(2):193-199, 1991). The cDNAs encoding CA89 and CA65 were cloned as blunt-end fragments into the psub-CAG-WPRE plasmid, and the recombinant AAV viruses (AAV.CA89 and AAV.CA65, AAV serotype 2) were produced as described in Karkkainen *et al., Proc. Natl. Acad. Sci. USA*, 98(22):12677-12682 (2001). The cDNAs encoding CA89 and CA65 were also cloned into the pAdBglII vector (AdCA89 and AdCA65), and recombinant adenoviruses were produced as described in Laitinen *et al., Hum. Gene Ther.*, 9(10):1481-1486, 1998. NCI-H460-LNM35 cells (Kozaki *et al., Cancer Res.*, 60(9):2535-2540, 2000) were used for expression analysis. These cells were maintained in RPMI1640 medium with supplements (2

mM L-glutamine, penicillin 100 U/ml, streptomycin 100 μg/ml, and 10% fetal bovine serum) as above and were infected with AAV.CAG.VEGFR-3-Ig viruses (MOI 2000), or adenoviruses (MOI 50). Expression of the recombinant proteins were examined by metabolic labeling, immunoprecipitation followed by SDS-PAGE analysis as described above.

Adenoviruses (AdCA89 or AdCA65, approximately 3×10^8 pfu), or AAV viruses (AAV.CA89, AAV.CA65 or AAV.EGFP, approximately 1×10^{10} viral particles) were injected subcutaneously into mouse ears. Tissues were collected for analysis after two weeks with adenoviruses and three weeks with AAV viruses for histological analysis.

Fluorescent microlymphography. The functional lymphatic network in the ears was visualized by fluorescent microlymphography using dextran conjugated with fluorescein isothiocyanate (molecular weight: 2000 kDa, Sigma) that was injected intradermally into the ears. The lymphatic vessels were examined using a dissection microscope (LEICA MZFLIII).

Immunohistochemistry. For whole mount staining, tissues were fixed in 4% paraformaldehyde (PFA), blocked with 3% milk in PBS, and incubated with polyclonal antibodies against LYVE-1 (Prevo *et al., J. Biol. Chem.*, 276(22):19420-12930, 2001) and PECAM-1 (PharMingen) overnight at 4 °C. Alexa594 and Alexa488 conjugated secondary antibodies (Molecular Probes) were used for staining, and samples were then mounted with Vectashield (Vector Laboratories) and analysed with a Zeiss LSM510 confocal microscope. For staining of tissue sections, tissues were fixed in 4% PFA overnight at 4°C and paraffin sections (6 μm) were immunostained with anti-LYVE-1 and monoclonal antibodies against PECAM-1.

Results and Discussion

As discussed in Example 1, the heparin binding property of growth factors is important in the biological activities of those factors that bind heparin. The data shown in Example 1 demonstrated that the presence of a heparin binding domain have an enhanced heparin binding activity as compared to native VEGF-C, and they are biologically active. The following discussion further corroborates those findings.

Enhancement of receptor binding activity of recombinantly processed VEGF-C by addition of heparin binding domain. Analysis of the receptor binding profiles of the chimeric molecules showed that, similar to VEGF-CΔNΔC, both CA89 and CA65 bound to VEGFR-2, VEGFR-3, but not VEGFR-1 (Fig. 3B). Heparin binding forms of VEGF, containing the VEGF exon 7-encoded sequence, have been shown to bind to neuropilins, which have important roles in the development of the cardiovascular and lymphatic systems (Soker *et al., J. Biol. Chem.*, 271(10):5761-5767, 1996; Neufeld *et al., Trends Cardiovasc. Med.*, 12(1):13-19, 2002). In agreement with these data, both CA89 and CA65 bound to NP-1 and NP-2, whereas VEGF-CΔNΔC had a weak binding activity to NP-2 but did not bind to NP-1 (Fig. 3A).

Lymphangiogenic activity of VEGF-CΔNΔC is enhanced by heparin/neuropilin binding domain. To further characterize the biological functions of the chimeric proteins *in vivo*, the cDNAs encoding CA89 and CA65 were cloned into the pAdBglII vector (AdCA89 and AdCA65) for the generation of recombinant adenoviruses. Recombinant AAV (AAV.CA89 and AAV.CA65, serotype 2) were also produced to study the effect of longer-term expression of the chimeric molecules. Shown in Fig. 4 is the analysis of polypeptides produced via the AAV (Fig. 4A) and adenoviral (Fig. 4B) expression of CA89, CA65, VEGF-C and the VEGF-CΔNΔC.

For analysis of their in vivo vascular effects, adenoviruses encoding CA89, CA65, and VEGF-CΔNΔC were injected subcutaneously into the ears of nude mice. AdVEGF-C (full length/"prepro-VEGF-C") and AdLacZ viruses were used as positive and negative controls. Tissues were collected for whole mount immunostaining of lymphatic vessels (LYVE-1 antigen) and blood vessels (PECAM-1) within two weeks. Both CA89 and CA65 were shown to induce strong lymphangiogenesis in comparison with the LacZ control. While CA89 exerted a localized effect around the virus injection site, CA65 induced a widespread effect in a fashion similar to the full-length VEGF-C. This is in agreement with the differential distribution of the two chimeric molecules between pericellular matrix and fluid phases in culture. VEGF-CΔNΔC induced only a weak lymphangiogenic effect with some lymphatic sprouting from the pre-existing lymphatic vessels. There was no

angiogenic effect observed with the heparin binding chimeric molecules, VEGF-CΔNΔC or full length VEGF-C in comparison with the control.

Both CA89 and CA65 delivered by the recombinant AAV viruses also induced strong lymphangiogenesis when compared with the control involving AAV.EGFP. However, the effects observed with AAV vectors were seen only around the ear muscles, as AAV viruses mainly transduce muscle and neurons (Daly, *Methods Mol. Biol.*, 246:157-165, 2004). The lymphatic vessels grew along the muscle fibers that were transduced with AAV.EGFP. These data indicate that by use of a vector/tissue-specific promoter and a heparin-binding growth factor, one can achieve a more defined localization of growth factor expression in a given tissue, and therefore minimize the danger of obtaining aberrant side effects from other sites.

However, analysis by microlymphography showed that the lymphatic vessels generated in the mice receiving CA89 or CA65 via viral vectors were leaky compared with the control. Similar findings have been reported for vessels generated with full-length VEGF-C. A combination of CA89 or CA65 with other molecules such as Ang-1 is contemplated for the optimal induction of functional lymphatic vessels.

In histological sections from the AAV.CA89 treated mice, many LYVE-1-positive vessel-like structures were observed in regions close to cartilage where the ear muscles are located, whereas only a few lymphatic vessels were found in corresponding sections from the control mice. PECAM-1, a panendothelial marker for blood and lymphatic vessels, also detected more vessels in the sections from the AAV.CA89 treated mice. Similarly, many LYVE-1-positive vessel-like structures, often in clusters close to the cartilage, were found in the AAV.CA65 treated mice. In contrast, fewer lymphatic vessels were observed in the control mice.

In summary, these experiments show the lymphangiogenic and/or angiogenic properties of VEGF-C short form in the presence and absence of a heparin binding property. Chimeric proteins made of the signal sequence and the VEGF homology domain (VHD) of VEGF-C, and the C-terminal domain of VEGF165 or VEGF189 isoforms containing heparin and neuropilin1 binding sequences (named CA89 and CA65) were studied. CA65 was secreted and released into the supernatant, but CA89 was only released if heparin was included in the culture medium. Analysis

of the receptor binding profiles of the chimeric molecules showed that they retained VEGFR-2 and VEGFR-3 binding and activation and in addition are expected to bind to NP-1, whereas the VEGF-C short form did not retain these binding activities. In vivo expression of the chimeric proteins delivered via adenoviral or associated virus vectors demonstrated that they induced strong lymphangiogenesis in a mouse ear model, whereas significant angiogenic activity was not observed. The enhanced lymphangiogenic activity may result from the increase of its bioavailability and/or neuropilin binding property.

Example 3
Methods of Using the Chimeric Polypeptides

The heparin binding VEGFR-3 binding ligands of the invention have utility in any and all indications for which VEGF-C and/or VEGF-D are useful, as well as additional indications for which these native VEGFR-3 ligands have shown limited or no efficacy. CA89 and CA65 are only two specific exemplary embodiments of the class of chimeric polypeptides of the present invention.

The activity of chimeric polypeptides of the present invention can be demonstrated in any of a number of assays. Examples of some of these assays are discussed below. To assess comparative/relative activities, these assays are conducted in parallel with molecules of the invention and with VEGF-A, VEGF-B, VEGF-C, VEGF-C_{156} mutants or fragments of a molecule of the invention containing only the VEGFR-3 binding domain or only the heparin binding domain.

Receptor Binding Assays

In a first battery of assays, one determines the receptor binding activity of the chimeric polypeptides of the present invention. It will be appreciated that such binding assays can be performed with any form of naturally occurring VEGF receptors that retain the ability to bind their respective ligands, including but not limited to whole cells that naturally express a receptor or that have been recombinantly modified to express the receptor; truncated, solubilized extracellular ligand binding domains of receptors; fusions comprising receptor extracellular domains fused to other proteins such as alkaline phosphatase (e.g., VEGF-R-2-AP described in Cao *et al., J. Biol. Chem. 271*:3154-62 (1996)) or immunoglobulin

sequences; and fusions comprising receptor extracellular domains fused to tag sequences (e.g., a polyhistidine tag) useful for capturing the protein with an antibody or with a solid support; and receptor extracellular domains chemically attached to solid supports such as CNBr-activated Sepharose beads. Exemplary receptor binding assays may be performed according to the method set forth in Example 3 of *e.g.*, U.S. Patent Application No. 09/795,006, and WO 01/62942, each incorporated herein by reference.

Analysis of receptor activation or inhibition by the chimeric VEGF proteins

In another set of assays, the chimeric polypeptides of the present invention are evaluated for therapeutic applications where either activation or inhibition of one or more VEGF receptors is desired. For example, a candidate chimeric protein can be added to stable cell lines expressing a particular VEGF receptor whose activation is necessary for cell survival. Survival of the cell line indicates that the candidate chimeric polypeptide protein is able to bind and activate that particular VEGF receptor. On the other hand, death of the cell line indicates that the candidate chimeric polypeptide fails to activate the receptor. Exemplary examples of such cell survival assays have been described in International Patent Publication No. WO 98/07832 and in Achen *et al., Proc Natl Acad Sci USA* 95:548 553 (1998), incorporated herein by reference. This assay employs Ba/F3 NYK EpoR cells, which are Ba/F3 pre B cells that have been transfected with a plasmid encoding a chimeric receptor consisting of the extracellular domain of VEGFR-2 and the cytoplasmic domain of the erythropoietin receptor (EpoR). These cells are routinely passaged in interleukin-3 (IL-3) and will die in the absence of IL-3. However, if signaling is induced from the cytoplasmic domain of the chimeric receptor, these cells survive and proliferate in the absence of IL-3. Such signaling is induced by ligands which bind to the VEGFR-2 extracellular domain of the chimeric receptor. For example, binding of VEGF A or VEGF-D to the VEGFR-2 extracellular domain causes the cells to survive and proliferate in the absence of IL-3. Parental Ba/F3 cells which lack the chimeric receptor are not induced by either VEGF A or VEGF-D to proliferate in the absence of IL-3, indicating that the responses of the Ba/F3-NYK-EpoR cells to these ligands are totally dependent on the chimeric receptor.

Candidate chimeric polypeptides of the present invention can be tested for binding to the VEGFR-2 extracellular domain and subsequent activation of the chimeric receptor by assaying cell survival in the absence of IL 3. On the other hand, chimeric polypeptides that interfere with the binding of VEGFR-2 ligands, such as VEGF-A or VEGF-D, to the extracellular domain, or with the activation of the cytoplasmic domain, will cause cell death in the absence of IL-3.

Typically, in these assays, cells are cultured in the presence of IL-3 until required, then washed three times in phosphate buffered saline (PBS), resuspended in IL-3-free cell culture medium (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with fetal calf serum (10%), L-glutamine (1%), geneticin (1mg/ml), streptomycin (100μg/ml) and penicillin (60μg/ml)), and replated in 72-well culture plates (Nunc, Denmark) at a density of approximately 1000 cells/well. To assay for receptor activity, candidate chimeric polypeptides are added to culture wells at final concentrations of 10-10 to 10-5 M and incubated for 1 hour at 37°C in 10% CO_2. For assaying the ability of the candidate chimeric polypeptides to inhibit activation of the VEGFR-2/EpoR receptor, recombinant VEGF A or VEGF-D is added to the chimeric polypeptide-containing wells at a concentration to produce near-maximal survival of the Ba/F3 NYK EpoR cells (typically 500ng/ml). Positive control cultures contain either VEGF-A or VEGF-D supernatant alone and negative control cultures contain neither chimeric polypeptide nor growth factor. Cells are then grown in culture for 48 hours, after which time a solution of 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 500μg/ml) is added to the cultures, and incubated for another 30 minutes. MTT is converted to a blue formazan product by mitochondria, thus staining living cells blue. Surviving blue cells in experiments where either activation (chimeric polypeptide alone) or inhibition (chimeric polypeptide + VEGF-A or VEGF-D) was assayed are counted under a microscope with inverted optics (100X magnification) and compared to cell survival in the positive control (VEGF-A or VEGF-D only) wells. Cell survival is normalized such that survival in negative controls is set to 0 (typically no viable cells were seen in negative controls), while survival in positive controls is set to 100% (typically 300-400 cells/well).

Typically, data is analyzed by one way analysis of variance (ANOVA), with a Bonferroni multiple comparisons test carried out post-hoc to test for

differences between individual cultures of chimeric polypeptide alone (to assay binding and activation of the receptor), or chimeric polypeptide + VEGF-A or VEGF-D (to assay inhibition of receptor activation), with VEGF-A or VEGF-D alone (positive control).

As described in Example 1, repetition of the same assay using cells transfected with different chimeric receptors (e.g., VEGFR-3/EpoR) allows screening for activation of different VEGFRs.

VEGFR-2 (KDR) and VEGFR-3 (Flt4) autophosphorylation assays.

As an alternative indicator of activity, the ability of a chimeric polypeptide of the invention to stimulate autophosphorylation of a particular VEGF receptor can also be examined. A candidate chimeric polypeptide is added to cells expressing a particular VEGF receptor. The cells are then lysed and immunoprecipitated with anti VEGF receptor antiserum and analyzed by Western blotting using anti phosphotyrosine antibodies to determine chimeric polypeptide induced phosphorylation of the VEGF receptor.

An expression vector comprising a polynucleotide encoding a chimeric VEGF polypeptide of the invention is transfected into an appropriate host cell (e.g., 293 EBNA cells using a calcium phosphate transfection method. About 48 hours after transfection, the growth medium of the transfected cells is changed (e.g., to DMEM medium lacking fetal calf serum) and the cells are incubated (e.g., for 36 more hours) to provide a conditioned medium. Heparin may be included in the culture medium to improve release of recombinant chimeric polypeptides into the medium as described in Example 1. The conditioned medium is collected and centrifuged at 5000 x g for 20 minutes, and the supernatant is concentrated.

The concentrated conditioned media is used to stimulate cells expressing a VEGF receptor. For example, PAE-KDR cells (Pajusola *et al., Oncogene*, 9:3545 55 (1994); Waltenberger *et al., J. Biol. Chem.*, 269: 26988 26995 (1994)) are grown in Ham's F12 medium-10% fetal calf serum (FCS), or confluent NIH 3T3 cells expressing VEGFR-3 are grown in DMEM medium. The cells are starved overnight in DMEM medium or Ham's F12 supplemented with 0.2% bovine serum albumin (BSA), and then incubated for 5 minutes with the unconcentrated, 2

fold, 5 fold, and/or 10 fold concentrated conditioned media. Recombinant human VEGF-A or VEGF-C and conditioned media from mock transfected cells are exemplary controls. In addition to conditional media, purified chimeric polypeptide can be employed in this or other assays described herein.

After stimulation with conditioned media, the cells are washed twice with ice cold Tris-Buffered Saline (TBS) containing 100 mM sodium orthovanadate and lysed in RIPA buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 U/ml aprotinin and 1 mM sodium orthovanadate. The lysates are sonicated, clarified by centrifugation at 16,000 x g for 20 minutes and incubated for 3-6 hours on ice with 3-5 µl of antisera specific for VEGFR-3 or VEGFR-2. Immunoprecipitates are bound to protein A-Sepharose, washed three times with RIPA buffer containing 1mM PMSF, 1mM sodium orthovanadate, washed twice with 10 mM Tris-HCl (pH 7.4), and subjected to SDS-PAGE using a 7% gel. Polypeptides are transferred to nitrocellulose by Western blotting and analyzed using PY20 phosphotyrosine-specific monoclonal antibodies (Transduction Laboratories) or receptor-specific antiserum and the ECL detection method (Amersham Corp.).

The ability of a chimeric polypeptide to stimulate autophosphorylation (detected using the anti phosphotyrosine antibodies) is scored as stimulating the receptor. The level of stimulation observed for various concentrations of chimeric polypeptide, relative to known concentrations of VEGF-A, VEGF-D or VEGF-C, provide an indication of the potency of receptor stimulation. Polypeptides that have been shown to bind the receptor, but are incapable of stimulating receptor phosphorylation, are scored as inhibitors. Inhibitory activity can be further assayed by mixing a known receptor agonist such as recombinant VEGF-A or VEGF-C with either media alone or with concentrated conditioned media, to determine if the concentrated conditioned media inhibits VEGF-A mediated or VEGF-C-mediated receptor phosphorylation.

Analysis of receptor binding affinities of chimeric polypeptides

The chimeric polypeptides of the present invention may bind more than one VEGFR. Assays may be performed to determine that receptor binding activity of these chimeric polypeptides. For such experiments, the chimeric

polypeptide may be expressed in an insect cell system, *e.g.*, SF9 cells, to eliminate contamination with endogenous VEGF-A found in mammalian cells. To measure the relative binding affinities of selected chimeric polypeptide, an ELISA-type approach is used. For example, to examine binding affinity for VEGFR-3, serial dilutions of competing VEGFR-3-IgG fusion proteins and a subsaturating concentration of the candidate chimeric polypeptide tagged with the *myc* epitope is added to microtitre plates coated with VEGFR-3, and incubated until equilibrium is established. The plates are then washed to remove unbound proteins. Chimeric polypeptide molecules that remain bound to the VEGFR-3 coated plates are detected using an anti-*myc* antibody conjugated to a readily detectable label *e.g.,* horseradish peroxidase. Binding affinities (EC_{50}) can be calculated as the concentration of competing VEGFR-IgG fusion protein that results in half-maximal binding. These values can be compared with those obtained from analysis of VEGF-A or VEGF-C to determine changes in binding affinity of one or more of the VEGFRs. Similarly, binding to VEGFR-2 is accomplished by using a VEGFR-2-IgG fusion protein, and binding to VEGFR-1 is determined using a VEGFR-1-IgG fusion protein.

Assays for neuropilin binding.

Recent results indicate that NRP-1 is a co-receptor for $VEGF_{165}$ binding, forming a complex with VEGFR-2, which results in enhanced $VEGF_{165}$ signaling through VEGFR-2, over $VEGF_{165}$ binding to VEGFR-2 alone, thereby enhancing the biological responses to this ligand (Soker et al., Cell 92: 735-45. 1998). A similar phenomenon may apply to VEGF-C signaling via possible VEGFR-3/NRP-2 receptor complexes. The compositions of the present invention are tested using neuropilin binding assays. Exemplary such assays are described in detail in e.g., U.S. Patent Application No. 10/669,176, filed September 23, 2003, U.S. Patent No. 6,428,965 and 6,515,105.

Such assays may employ cells transformed with expression constructs that encode neuropilins. Antibodies and reagents that can be used in neuropilin binding assays are well known to those of skill in the art. See for example, Sema3A-AP which recognizes neuropilin. Competitive binding assays using Sema3 AP and the compositions of the invention will reveal whether the compositions described

herein possess neuropilin binding activity. The assays may also use a cell-free complex to determine the binding of the chimerical molecules of the invention competing with binding of VEGF-B_{167}, VEGF-C, VEGF-D or processed VEGF-B186 to a receptor. Such a cell-free complex would comprise at least one neuropilin receptor molecule, for example soluble NP-1 (sNP-1) and the composition to be tested. The sNP-1 is defined as a non-membrane bound protein as well as a portion of the receptor, such as the extracellular domain or the ligand-binding fragment of NP-1.

Assays for endothelial cell migration in collagen gel.

Both VEGF-A and VEGF-C stimulate endothelial cell migration in collagen gel. The chimeric polypeptides of the invention are examined to determine if they are also capable of stimulating endothelial cell migration in collagen gel, thus providing another indicia of biological activity. Exemplary experiments of such cell migration assays have been described in International Patent Publication No. WO 98/33917, incorporated herein by reference. Briefly, bovine capillary endothelial cells (BCE) are seeded on top of a collagen layer in tissue culture plates. Conditioned media from cells transfected with an expression vector producing the candidate chimeric polypeptide is placed in wells made in collagen gel approximately 4mm away from the location of the attached BCE cells. The number of BCE cells that have migrated from the original area of attachment in the collagen gel towards the wells containing the chimeric polypeptide is then counted to assess the ability of the chimeric polypeptide to induce cell migration.

BCE cells (Folkman *et al., Proc. Natl. Acad. Sci. (USA), 76:*5217-5221 (1979)) are cultured as described in Pertovaara *et al., J. Biol. Chem., 269:*6271-74 (1994). Collagen gels are prepared by mixing type I collagen stock solution (5 mg/ml in 1 mM HCl) with an equal volume of 2x MEM and 2 volumes of MEM containing 10% newborn calf serum to give a final collagen concentration of 1.25 mg/ml. Tissue culture plates (5 cm diameter) are coated with about 1 mm thick layer of the solution, which is allowed to polymerize at 37°C. BCE cells are seeded atop this layer.

For the migration assays, the cells are allowed to attach inside a plastic ring (1 cm diameter) placed on top of the first collagen layer. After 30 minutes, the

ring is removed and unattached cells are rinsed away. A second layer of collagen and a layer of growth medium (5% newborn calf serum (NCS)), solidified by 0.75% low melting point agar (FMC BioProducts, Rockland, ME), are added. A well (3 mm diameter) is punched through all the layers on both sides of the cell spot at a distance of 4 mm, and media containing a chimeric VEGF polypeptide (or media alone or media containing VEGF-A or VEGF-C to serve as controls) is pipetted daily into the wells. Photomicrographs of the cells migrating out from the spot edge are taken, e.g., after six days, through an Olympus CK 2 inverted microscope equipped with phase-contrast optics. The migrating cells are counted after nuclear staining with the fluorescent dye bisbenzimide (1 mg/ml, Hoechst 33258, Sigma).

The number of cells migrating at different distances from the original area of attachment towards wells containing media conditioned by the non-transfected (control) or transfected (mock; chimeric polypeptide; VEGF-C; or VEGF-A) cells are determined 6 days after addition of the media. The number of cells migrating out from the original ring of attachment are counted in five adjacent 0.5 mm x 0.5 mm squares using a microscope ocular lens grid and 10x magnification with a fluorescence microscope. Cells migrating further than 0.5 mm are counted in a similar way by moving the grid in 0.5 mm steps.

The ability of a chimeric polypeptide to induce migration of BCE cells is indicative of receptor agonist activity. The number of migrating cells in the presence of a chimeric polypeptide versus a similar concentration of VEGF-A or VEGF-C provides an indication of the potency of agonist activity. Polypeptides that have been shown to bind the receptors expressed on BCE cells, but are incapable of stimulating migration, are scored as potential inhibitors. Inhibitory activity can be further assayed by mixing a known receptor agonist such as recombinant VEGF-A or VEGF-C with either media alone or with concentrated conditioned media, to determine if the concentrated conditioned media inhibits VEGF-A-mediated or VEGF-C-mediated BCE migration.

Assay for induction of vascular permeability

Both VEGF-A and VEGF-C are capable of increasing the permeability of blood vessels. The chimeric polypeptides of the invention are assayed to determine which of these proteins possess this biological activity and which inhibit it. For

example, vascular permeability assays according to Miles and Miles, *J. Physiol 118*:228-257 (1952), incorporated herein in its entirety, are used to analyze the chimeric polypeptide . Briefly, following intravenous injection of a vital dye, such as pontamine sky blue, animals such as guinea pigs are injected intradermally with a composition containing the candidate chimeric polypeptide being examined. For controls, media alone or media containing VEGF-A or VEGF-C is injected in the same manner. After a period of time, the accumulation of dye at the injection site on the skin is measured. Those chimeric polypeptides that increase permeability will result in greater accumulation of dye at the injection site as compared to those chimeric polypeptides that fail to induce vascular permeability.

In a variation of this assay, chimeric polypeptides that are suspected of being inhibitors of VEGF-A or VEGF-C are first mixed with VEGF-A or with VEGF-C at varying ratios (e.g., 50:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10) and the mixtures are injected intradermally into the animals. In this manner, the ability of the chimeric polypeptide to inhibit VEGF-A-mediated or VEGF-C-mediated vascular permeability is assayed.

Endothelial Cell Proliferation Assays

The mitogenic activity of the chimeric polypeptides can be examined using endothelial cell proliferation assays such as that described in Breier *et al., Dev 114*:521-532 (1992), incorporated herein in its entirety. The chimeric polypeptides are expressed in a mammalian cell line *e.g.*, COS cells. Culture supernatants are then collected and assayed for mitogenic activity on bovine aortic endothelial (BAE) cells by adding the supernatants to the BAE cells. After three days, the cells are dissociated with trypsin and counted using a cytometer to determine any effects of the chimeric polypeptide on the proliferative activity of the BAE cells. As negative controls, DMEM supplemented with 10% FCS and the conditioned media from untransfected COS cells or from COS cells transfected with vector alone can be used. Supernatants from cells transfected with constructs expressing proteins that have been shown to induce proliferation of BAE cells (*e.g.*, VEGF-A) can be used as a positive control.

Induction of *in vivo* growth of lymphatic and/or blood vessels in skin of transgenic mice

Experiments may be conducted in transgenic mice to analyze the specific effects of overexpression of chimeric polypeptides in tissues. The physiological effects *in vivo* provide an indication of receptor activation/inhibition profile and an indication of the potential therapeutic action of a chimeric polypeptide. In one variation, the human K14 keratin promoter which is active in the basal cells of stratified squamous epithelia [Vassar *et al., Proc. Natl. Acad. Sci. (USA), 86:*1563-1567 (1989)], is used as the expression control element in the recombinant chimeric polypeptide transgene. The vector containing the K14 keratin promoter is described in Vassar *et al., Genes Dev., 5:*714-727 (1991) and Nelson *et al., J. Cell Biol. 97:*244-251 (1983).

A DNA fragment containing the K14 promoter, chimeric polypeptide encoding cDNA, and K14 polyadenylation signal is isolated, and injected into fertilized oocytes of the FVB-NIH mouse strain. The injected zygotes are transplanted to oviducts of pseudopregnant C57BL/6 x DBA/2J hybrid mice. The resulting founder mice are then analyzed for the presence of the transgene by polymerase chain reaction of tail DNA using appropriate primers or by Southern analysis.

These transgenic mice are then examined for evidence of angiogenesis or lymphangiogenesis in the skin, such as the lymphangiogenesis seen in transgenic mice that overexpress VEGF-C [see International Publication WO98/33917]. Histological examination of K14-VEGF-C transgenic mice showed that in comparison to the skin of wildtype littermates, the dorsal dermis was atrophic and connective tissue was replaced by large lacunae devoid of red cells, but lined with a thin endothelial layer. These distended vessel-like structures resembled those seen in human lymphangiomas. The number of skin adnexal organs and hair follicles were reduced. In the snout region, an increased number of vessels was also seen.

Examination of the vessels in the skin of the transgenic mice using antibodies that recognize proteins specific for either blood or lymphatic vessels can further verify the identity of these vessels. Collagen types IV, XVIII [Muragaki *et al., Proc. Natl. Acad. Sci. USA, 92*: 8763-8776 (1995)] and laminin are expressed in

vascular endothelial cells while desmoplakins I and II (Progen) are expressed in lymphatic endothelial cells. See Schmelz *et al.*, *Differentiation, 57*: 97-117 (1994).

In addition, the chimeric molecules can be co-expressed with Ang-1 or PDGF-B in transgenic mice, to determine whether abnormalities of lymphatic vesels observed in K14-VEGF-C mice can be corrected.

Assays for Determining Modulation of Myelopoiesis

Overexpression of VEGF-C in the skin of K14-VEGF-C transgenic mice correlates with a distinct alteration in leukocyte populations [see International Publication WO98/33917]. Notably, the measured populations of neutrophils were markedly increased in the transgenic mice. The effects of the chimeric polypeptides on hematopoiesis can be analyzed using fluorescence-activated cell sorting analysis using antibodies that recognize proteins expressed on specific leukocyte cell populations. Leukocyte populations are analyzed in blood samples taken from the F1 transgenic mice described above, and from their non-transgenic littermates. Alterations in leukocyte populations has numerous therapeutic indications, such as stimulating an immune response to pathogens, recovery of the immune system following chemotherapy or other suppressive therapy, or in the case of inhibitors, beneficial immunosuppression (e.g., to prevent graft-versus-host-disease or autoimmune disorders.) Use of molecules of the invention for these therapeutic indications is specifically contemplated.

Assays to determine effect on growth and differentiation of human CD34+ progenitor cells *in vitro*

Addition of VEGF-C to cultures of cord blood CD34+ cells induces cell proliferation. Co-culture of GM-CSF, IL-3, GM-CSF + IL-3, or GM-CSF + SCF with VEGF-C leads to an enhancement of proportions of myeloid cells [see International Publication WO98/33917]. Chimeric polypeptides of the invention can also be examined for their ability to induce growth of CD34+ progenitor cells *in vitro*. Human CD34+ progenitor cells (HPC, 10 x 103) are isolated from bone marrow or cord blood mononuclear cells using the MACS CD34 Progenitor cell Isolation Kit (Miltenyi Biotec, Bergish Gladbach, Germany), according to the instructions of the

manufacturer and cultured in RPMI 1640 medium supplemented with L-glutamine (2.5 mM), penicillin (125 IE/ml), streptomycin (125 μg/ml) and pooled 10 % umbilical cord blood (CB) plasma at 37 °C in a humidified atmosphere in the presence of 5% CO2 for seven days, with or without chimeric polypeptide at concentrations ranging from 10 ng/ml to 1 μg/ml. After seven days, total cell number is evaluated in each culture.

The co-stimulatory effect of chimeric polypeptides in cultures either supplemented with recombinant human stem cell factor (rhSCF, 20 ng/ml PreproTech, Rocky Hill, NY) alone or a combination of granulocyte macrophage colony stimulating factor (rhGM-CSF, 100 ng/ml, Sandoz, Basel, Switzerland) plus SCF can also be examined. Experiments can also be conducted to analyze the co-stimulatory effects of chimeric polypeptide on total cell yields of serum-free cultures of CB CD34+ HPC cells supplemented with either GM-CSF alone, IL-3 (rhIL-3, 100 U/ml, Behring AG, Marburg, Germany) alone; or a combination of GM-CSF plus IL-3.

Cells from the (7 day) plasma-supplemented cultures described above are also analyzed for the expression of the early granulomonocytic marker molecules lysozyme (LZ) and myeloperoxidase (MPO) as well as the lipopolysaccharide (LPS) receptor CD14 using immunofluorescence.

In another series of experiments, CD34+ cells are cultured in medium supplemented with 50 ng/ml M-CSF, with or without 100 ng/ml chimeric polypeptide, for seven days. After seven days, the cultures were analyzed to determine the percentages of CD34+ cells and mean fluorescence intensity.

Analysis of Chimeric Polypeptides using CAM assays

The choroallantoic membrane (CAM) assay described in e.g., Oh *et al., Dev Biol 188* :96-109 (1997), incorporated herein in its entirety, is a commonly used method to examine the *in vivo* effects of angiogenic factors. Using this assay, VEGF growth factors including both VEGF-A and VEGF-C have been shown to induce the development of blood vessels [Oh *et al., Dev Biol 188*:96-109 (1997)]. Thus, this method can be used to study the angiogenic properties of the chimeric polypeptide.

Briefly, on day 4 of development, a window is cut out into the eggshell of chick or quail eggs. The embryos are checked for normal development, the window in the eggshell is sealed with cellotape, and the eggs are incubated until day 13 of development. Approximately 3.3 μg of chimeric polypeptide dissolved in 5 μl of distilled water is added to Thermanox coverslips (Nunc, Naperville, IL), which have been cut into disks with diameters of approximately 5 mm, and air dried. Disks without added protein are used as controls. The dried disks are then applied on the chorioallantoic membrane (CAM) of the eggs. After 3 days, the disks are removed and fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. The fixed specimens are photographed and embedded in Epon resin (Serva, Germany) for semi- (0.75 μm) and ultrathin (70 nm) sectioning. Both semi- and ultrathin sections are cut using an Ultracut S (Leika, Germany). Ultrathins sections are analyzed by an EM 10 (Zeiss, Germany). Specimens are then analyzed for evidence of growth of new capillaries, which would indicate that the chimeric polypeptide being examined is capable of stimulating angiogenesis.

Angiogenesis in Tissue Ischemia

Utility of chimeric polypeptides of the invention in treating ischemic tissue, such as limb ischemia due to insufficient circulation, is analyzed using recognized assays. The efficacy of the chimeric polypeptides in such indications may be determined using model for such ischemia, such as for example a rabbit model for ischemia has previously been described (Bauters *et al., Am J. Physiol.* 267:H1263-1271, 1996; Pu *et al., J. Invest. Surgery*, 7:49-60, 1994). These animals are anesthetized and the femoral artery of on hindlimb is excised from its proximal origin as a branch of the external iliac artery to the point where it bifurcates into the saphenous and popliteal arteries. As a result of this procedure, the blood flow to the ischemic limb is dependent on collateral vessels originating from the internal iliac artery Takeshita *et al., Circulation,* 90:II-228-II-234, 1994). The animal is allowed a 10-day post-operative recovery period. During this period, endogenous collateral vessels develop. After the recovery period, the baseline physiological parameters, such as blood pressure, intravascular blood flow, iliac angiography and capillary vessel density is determined. Methods for determining these baseline physiological

characteristics are detailed in Witzenbichler *et al.*, (*Am. J. Path.* 153:381-394, 1998). Lymphatic vessels also are analyzed as described in Example 2.

After obtaining the baseline physiological characteristics of the animal, the model animal is treated with an intra-arterial bolus of a chimeric polypeptide of the present invention. Preferably, the bolus comprises the equivalent of 500 μg of VEGF-C in an appropriate volume, e.g., 3 ml, of phosphate buffered saline (PBS) containing 0.1% rabbit serum albumin (RSA). The chimeric protein is administered over a period of 1 to 5 minutes through a catheter positioned in the internal iliac artery of the ischemic limb. The catheter is then washed with an equal volume of PBS containing RSA. The physiological parameters discussed above are then monitored at suitable intervals after administration of the chimeric polypeptides.

In an alternative embodiment, the ischemic model is treated using gene therapy with either naked DNA comprising polynucleotides that encode the chimeric polypeptides of the present invention or, preferably, gene therapy vectors described herein that encode a chimeric polypeptide of the present invention. Adenoviral gene therapy vectors are particularly preferred. In such gene therapy embodiments, the internal iliac artery of the ischemic limb of the animal is transfected with the naked DNA or the adenoviral or other gene therapy vector using e..g., a 2.0mm balloon catheter (Slider with Hydroplus, Boston Scientific, MA). The angioplasty balloon is preferably prepared ex vivo by first advancing the deflated balloon througha Teflon sheath (Boston Scientific) and applying the gene therapy composition to the layer of hydrogel coating the external surface of the inflated balloon. The balloon is then retracted back into its protective sheath. The sheath and the angioplasty catheter are introduced via the right carotid artery and advanced to the lower abdominal aorta using an appropriate guide-wire. The balloon catheter is advanced to the internal iliac artery of the ischemic limb and inflated to administer the gene therapy composition locally at the ischemic limb. The balloon catheter is then deflated and withdrawn.

The above methods may be performed with controls that comprise no VEGF-related composition and other controls which comprise VEGF-C, VEGF-D or even VEGF-A.

The above studies are described with respect to a rabbit model for ischemia. Similar studies may be conducted in models of ischemic heart disease, such

as those described by Kastrup *et al.*, (*Curr. Gene Ther.*, 3(3):197-206, 2003), and Khan *et al.*, (*Gene Ther.* 10(4):258-91, 2003).

A further indication for the compositions of the present invention may be demonstrated *in vivo* in rabbit restenosis models, to demonstrate the efficacy of the compositions for the prevention of post-angioplasty restenosis. The animal models typically are rabbits, but other mammals may be tested. A first group of rabbits is fed a 0.25 % cholesterol diet for two weeks, then subjected to balloon denudation of the aorta, then subjected three days later to the therapeutic compositions to be tested. Animals are sacrificed 2 or 4 weeks after the initiation of therapy. The compositions to be tested include VEGF-C, or VEGF-D or chimeric compositions of the invention that comprise VEGF-C or VEGF-D, either alone or in combination with a PDGF inhibitor (for example an α-PDGF-A antibody; α-PDGF-B antibody, α-PDGF-C antibody, α-PDGF-D antibody, a α-PDGFR-alpha antibody or a α-PDGFR-beta antibody or a short interfering RNA molecule directed to one or more of these targets) or with one or more other smooth muscle cell growth inhibitors. Polypeptide therapy or gene therapy is contemplated. As a gene therapy control, the vector of choice carries the LacZ gene.

In the first group of rabbits, the whole aorta, beginning from the tip of the arch, is denuded using a 4.0 F arterial embolectomy catheter (Sorin Biomedical, Irvine, CA). The catheter is introduced via the right iliac artery up to the aortic arch and inflated, and the aorta is denuded twice.

Three hours before sacrifice, the animals are injected intravenously with 50 mg of BrdU dissolved in 40 % ethanol. After the sacrifice, the aortic segment where the gene transfer had been performed is removed, flushed gently with saline, and divided into five equal segments. The proximal segment is snap frozen in liquid nitrogen and stored at -70°C. The next segment is immersion-fixed in 4 % paraformaldehyde / 15 % sucrose (pH 7.4) for 4 hours, rinsed in 15 % sucrose (pH 7.4) overnight, and embedded in paraffin. The medial segment is immersion-fixed in 4 % paraformaldehyde / phosphate buffered saline (PBS) (pH 7.4) for 10 minutes, rinsed 2 hours in PBS, embedded in OCT compound (Miles), and stored at -70°C. The fourth segment is immersion-fixed in 70 % ethanol overnight and embedded in paraffin. The distal segment is directly stained for β-galactosidase activity in X-GAL staining solution at +37°C for 16 hours, immersion-fixed in 4 % paraformaldehyde /

15 % sucrose (pH 7.4) for 4 hours, rinsed in 15 % sucrose overnight, and embedded in paraffin. Paraffin sections are used for immunocytochemical detection of smooth muscle cells (SMC), macrophages, and endothelium. BrdU-positive cells are detected according to manufacturer's instructions. Morphometric analysis performed using haematoxylin-eosin stained paraffin sections using image analysis software. Intima/media (I/M) ratio is used as a parameter for intimal thickening.

Histological analysis of the balloon-denuded mice is taken. Compositions that are effective at inhibiting restenosis will reveal that control groups (i.e., those groups without the compositions that comprise the VEGF-C related compositions) have an I/M ratio of that is higher than the ratio from those animals treated with the VEGF-C-based therapeutic compositions.

The BrdU labeling will permit analysis of smooth muscle cell proliferation in treated versus control animals. SMC proliferation is expected to be reduced in the treated population. A more detailed description of assays and compositions for treating restenosisis contained in international application no. PCT/US99/24054, published as WO 00/24412 incorporated herein by reference in its entirety.

It should be understood that the foregoing description relates to preferred embodiments of the invention and equivalents and variations that will be apparent to the reader are also intended as aspects of the invention. The references cited herein throughout, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are all specifically incorporated herein by reference.

CLAIMS

What is claimed is:

1. A compound comprising the formula **X-B-Z** or **Z-B-X**,

wherein **X** binds Vascular Endothelial Growth Factor Receptor 3 (VEGFR-3) and comprises an amino acid sequence at least 90% identical to a VEGFR-3 ligand selected from the group consisting of:

(a) the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2;

(b) fragments of (a) that bind VEGFR-3;

(c) the prepro-VEGF-D amino acid sequence set forth in SEQ ID NO: 4; and

(d) fragments of (c) that bind VEGFR-3;

wherein **Z** comprises a heparin-binding amino acid sequence; and

wherein **B** comprises a covalent attachment linking **X** to **Z**.

2. The compound of claim 1, wherein **X** comprises an amino acid sequence at least 95% identical to a VEGFR-3 ligand selected from the group consisting of:

(a) the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2; and

(b) fragments of (a) that bind VEGFR-3.

3. The compound of claim 1, wherein when **X** comprises an amino acid sequence at least 95% identical to a VEGFR-3 ligand selected from the group consisting of:

(a) the prepro-VEGF-D amino acid sequence set forth in SEQ ID NO: 4; and

(b) fragments of (a) that bind VEGFR-3.

4. The compound of any one of claims 1-3, wherein the compound binds Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2).

5. The compound of any one of claims 1-4, wherein the heparin binding amino acid sequence comprises an amino acid sequence at least 90% identical to a sequence selected from the group consisting of:

(a) amino acids 142-165 of the $VEGF_{206}$ (SEQ ID NO: 5);

(b) amino acids 183 to 226 of the $VEGF_{206}$ (SEQ ID NO: 5);

(c) amino acids 142-165 (SEQ ID NO: 5) joined directly to amino acids 183-226 (SEQ ID NO: 5) of the $VEGF_{206}$;

(d) amino acids 142 to 226 of the $VEGF_{206}$ (SEQ ID NO: 5);

(e) amino acids 138 to 182 of the VEGF-B_{167} sequence set forth in SEQ ID NO: 8;

(f) amino acids 193 to 213 of the PlGF-3 sequence set forth in SEQ ID NO: 15;

(g) amino acids of 142 to 162 of the PlGF-2 sequence set forth in SEQ ID NO:69;

(h) fragments of (a) - (g) that bind heparin.

6. The compound of any one of claims 1-5, wherein **X-B-Z** or **Z-B-X**, is a polypeptide.

7. The compound of any one of claims 1-6, further comprising a signal peptide at the amino terminus of the polypeptide, wherein the signal peptide directs secretion of a polypeptide comprising **X-B-Z** or **Z-B-X** from a cell that expresses the polypeptide.

8. The compound of any one of claims 1-7, wherein **B** is selected from the group consisting of:

(a) a peptide bond; and

(b) a peptide linker up to 500 amino acids.

9. The compound of any one of claims 1-8, wherein **B** comprises a peptide bond that is cleavable by an agent that fails to cleave the amino acid sequence **X** that binds VEGFR-3.

10. The compound of claim 9, wherein peptide bond is cleaved by a protease.

11. The compound of claim 9, wherein **B** comprises an amino acid sequence that contains a protease cleavage site selected from the group consisting of a Factor Xa cleavage site, an enterokinase cleavage site, a thrombin cleavage site, a TEV cleavage site, and a PreScission cleavage site.

12. The compound of any one of claims 1-11, wherein **B** comprises an amino acid sequence of at least four amino acids from a VEGF-C or VEGF-D amino acid sequence, wherein the at least four amino acids are cleaved *in vivo* to separate an amino-terminal propeptide that includes the heparin binding amino acid sequence from a mature VEGF-C or VEGF-D protein.

13. The compound of any one of claims 1-7, wherein **B** is selected from the group consisting of a peptide bond and a peptide linker up to 50 amino cells in length.

14. The compound of any one of claims 1-2 and 4-13, wherein **X** comprises an amino acid sequence at least 95% identical to the prepro-VEGF-C

amino acid sequence set forth in SEQ ID NO: 2 or to a fragment thereof that binds VEGFR-3,

with the proviso that the cysteine corresponding to amino acid position 156 of SEQ ID NO: 2 has been deleted or replaced with an amino acid other than cysteine, and the resultant amino acid sequence binds VEGFR-3 but has reduced VEGFR-2 binding.

15. The compound of any one of claims 1-2 and 4-13, wherein **X** comprises an amino acid sequence identical to the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2 or to a fragment thereof that binds VEGFR-3.

16. The compound of any one of claims 1-2 and 4-13, wherein **X** comprises an amino acid sequence identical to the prepro-VEGF-C amino acid sequence set forth in SEQ ID NO: 2 or to a fragment thereof that binds VEGFR-3,

with the proviso that the cysteine corresponding to amino acid position 156 of SEQ ID NO: 2 has been deleted or replaced with an amino acid other than cysteine, and the resultant amino acid sequence binds VEGFR-3 but has reduced VEGFR-2 binding.

17. The compound of any one of claims 1 and 3-13, wherein **X** comprises an amino acid sequence identical to the prepro-VEGF-D amino acid sequence set forth in SEQ ID NO: 4 or to a fragment thereof that binds VEGFR-3.

18. The compound of any one of claims 1-17, wherein the compound further includes a peptide tag (e.g., a polyhistidine tag) to facilitate purification.

19. A composition comprising a compound of any one of claims 1-18 in a pharmaceutically acceptable carrier.

20. A polynucleotide comprising a nucleotide sequence that encodes a compound of any one of claims 1-18.

21. A polynucleotide of claim 20, wherein the polynucleotide further comprises a nucleotide sequence that encodes a signal peptide fused in-frame with the polypeptide.

22. A vector comprising a polynucleotide of claim 20 or 21.

23. An expression vector comprising a polynucleotide of claim 20 or 21 operably linked to an expression control sequence.

24. An expression vector of claim 23, wherein the expression control sequence is an endothelial cell specific promoter.

25. A vector of any one of claims 22-24, selected from the group consisting of replication deficient adenoviral vectors, adeno-associated viral vectors, and lentivirus vectors.

26. A composition comprising a polynucleotide of claim 20 or 21 and a pharmaceutically acceptable carrier, diluent or excipient.

27. A composition comprising a vector of any one of claims 22-24 and a pharmaceutically acceptable carrier, diluent or excipient.

28. A host cell transformed or transfected with a polynucleotide of claim 20 or 21.

29. A host cell transformed or transfected with a vector of any one of claims 22-24.

30. A host cell that expresses a compound of any one of claims 1-18.

31. A method of modulating the growth of mammalian endothelial cells or mammalian endothelial precursor cells, comprising contacting the cells with a composition comprising a member selected from the group consisting of:

(a) a polypeptide compound of any one of claims 1-18;

(b) a polynucleotide that encodes (a);

(c) an expression vector containing (b) operatively linked to an expression control sequence; and

(d) a cell transformed or transfected with (b) or (c) that expresses the polypeptide of (a).

32. A method of claim 31, wherein the contacting comprises administering the composition to a mammalian subject in an amount effective to modulate endothelial cell growth *in vivo*.

33. A method of claim 31 or 32, wherein the mammalian subject is a human.

34. A method according to any one of claims 31-33, wherein the subject has lymphedema.

35. A method of modulating the growth of mammalian hematopoietic progenitor cells, comprising contacting the cells with a composition comprising a member selected from the group consisting of:

(a) a polypeptide compound of any one of claims 1-18;

(b) a polynucleotide that encodes (a);

(c) an expression vector containing (b) operatively linked to an expression control sequence; and

(d) a cell transformed or transfected with (b) or (c) that expresses the polypeptide compound of (a).

36. A method for activation of VEGFR-3, comprising contacting cells that express VEGFR-3 with a composition comprising a compound of any one of claims 1-18.

37. A method of stimulating lymphangiogenesis in a mammal comprising contacting said mammal with, and/or administering to said mammal, a composition comprising a member selected from the group consisting of:

(a) a polypeptide compound of any one of claims 1-18;

(b) a polynucleotide that encodes (a);

(c) an expression vector containing (b) operatively linked to an expression control sequence; and

(d) a cell transformed or transfected with (b) or (c) that expresses the polypeptide compound of (a).

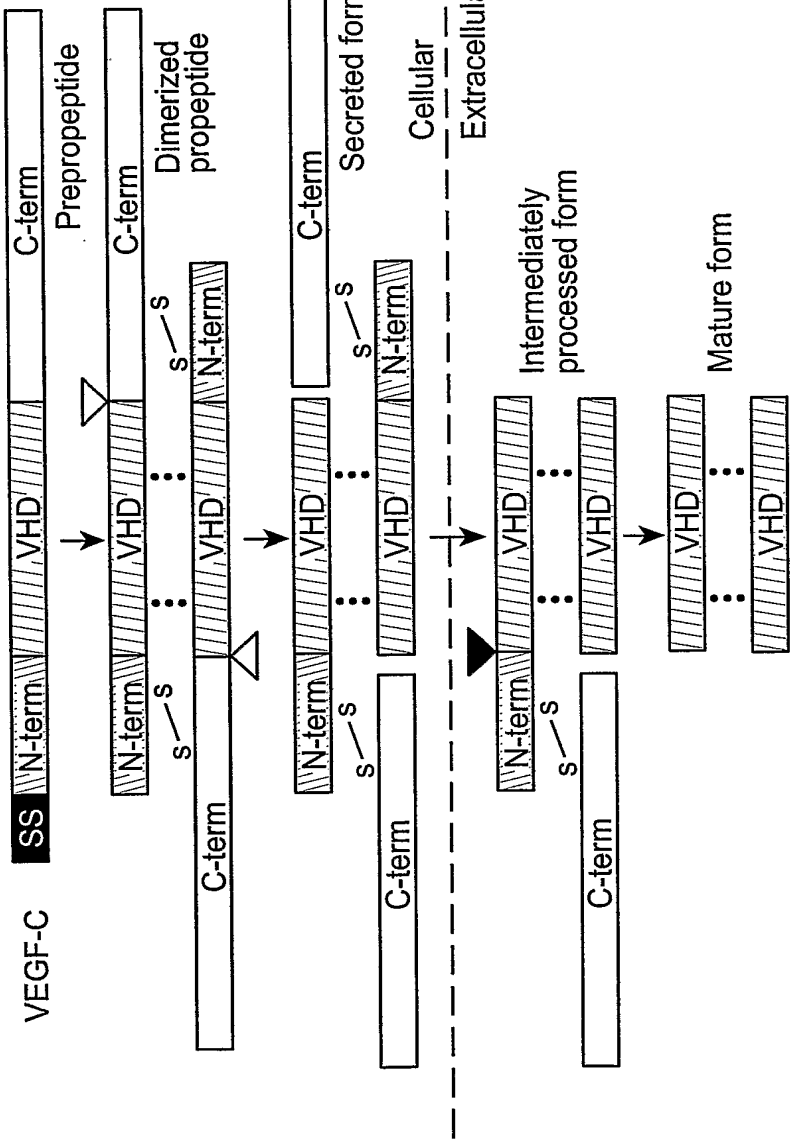
38. A method of stimulating angiogenesis in a mammal comprising contacting said mammal with a composition comprising a member selected from the group consisting of:

(a) a polypeptide compound of claim 4;

(b) a polynucleotide that encodes (a);

(c) an expression vector containing (b) operatively linked to an expression control sequence; and

(d) a cell transformed or transfected with (b) or (c) that expresses the polypeptide (a).


VEGF-C
SS
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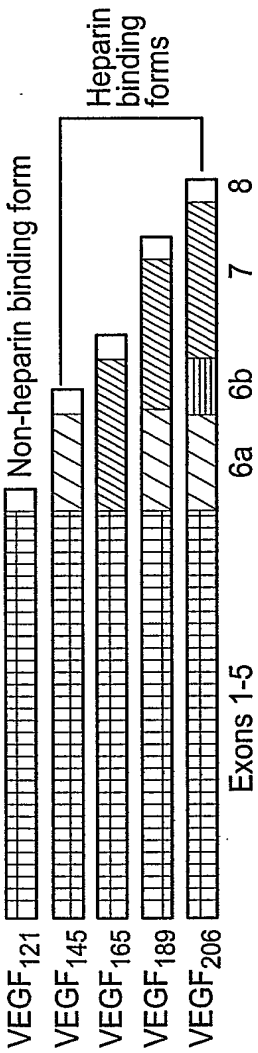
VHD
C-term
Prepropeptide
Dimerized propeptide
Secreted form
Cellular
Extracellular
Intermediately processed form
Mature form
S

FIG. 1A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Non-heparin binding form
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VEGF145
VEGF165
VEGF189
VEGF206
Exons 1-5
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6b
7
8

FIG. 1B

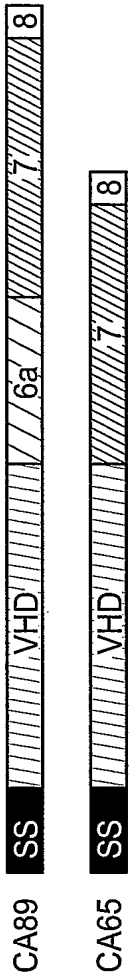
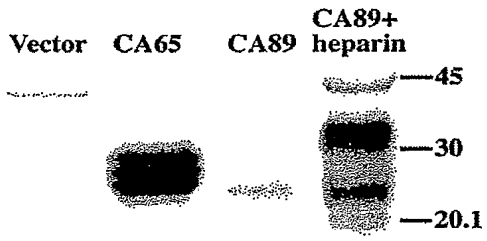

CA89
CA65
SS
VHD
6a
7
8

FIG. 1C



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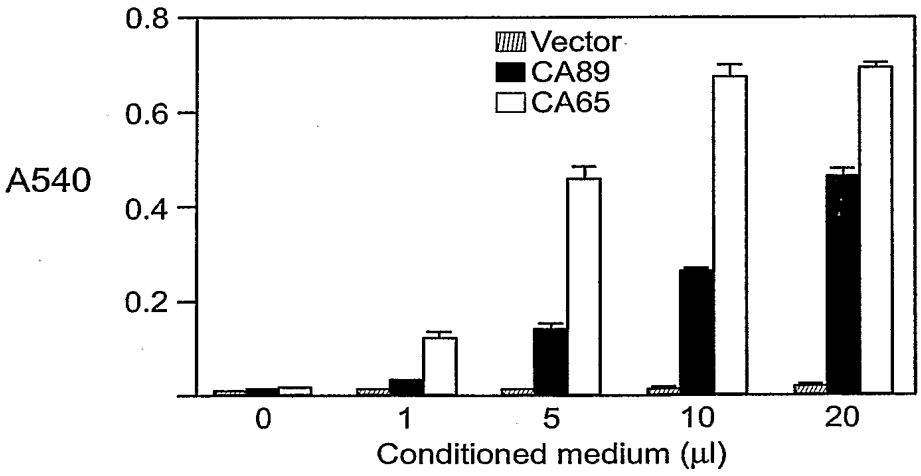
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Conditioned medium (µl)

FIG. 2

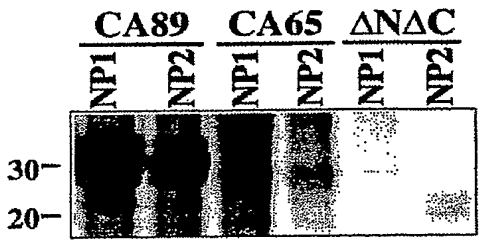
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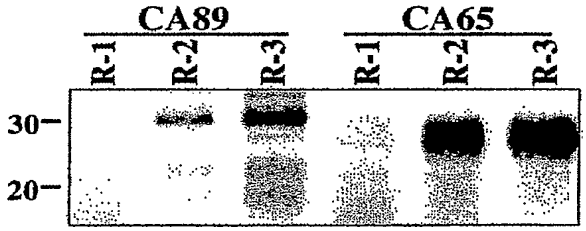
FIG. 3A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CA65
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R-2
R-3
R-1
R-2
R-3
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20

FIG. 3B

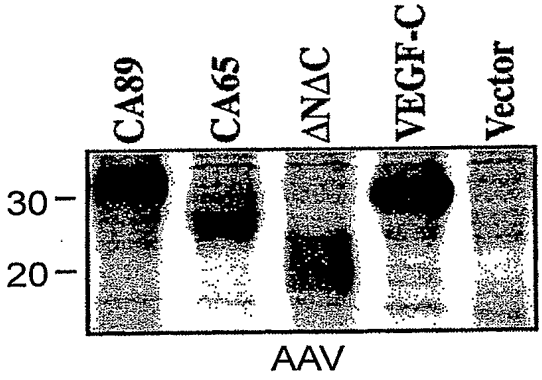

FIG. 4A

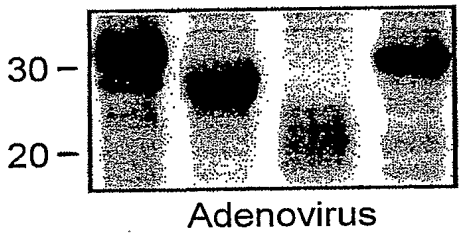

FIG. 4B

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ggaacgcgga gccccggacc cgctcccgcc gcctccggct cgcccagggg gggtcgccgg 240

gaggagcccg ggggagaggg accaggaggg gcccgcggcc tcgcaggggc gcccgcgccc 300

ccacccctgc ccccgccagc ggaccggtcc cccacccccg gtccttccac c atg cac 357
Met His
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tat gca agc aaa gat ctg gag gag cag tta cgg tct gtg tcc agt gta 549
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        85                  90                  95

ctc aac tca agg aca gaa gag act ata aaa ttt gct gca gca cat tat      693
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aat aca gag atc ttg aaa agt att gat aat gag tgg aga aag act caa      741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln
115                 120                 125                 130

tgc atg cca cgg gag gtg tgt ata gat gtg ggg aag gag ttt gga gtc      789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val
                135                 140                 145

gcg aca aac acc ttc ttt aaa cct cca tgt gtg tcc gtc tac aga tgt      837
Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys
            150                 155                 160

ggg ggt tgc tgc aat agt gag ggg ctg cag tgc atg aac acc agc acg      885
Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr
        165                 170                 175

agc tac ctc agc aag acg tta ttt gaa att aca gtg cct ctc tct caa      933
Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln
    180                 185                 190

ggc ccc aaa cca gta aca atc agt ttt gcc aat cac act tcc tgc cga      981
Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg
195                 200                 205                 210

tgc atg tct aaa ctg gat gtt tac aga caa gtt cat tcc att att aga     1029
Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg
                215                 220                 225

cgt tcc ctg cca gca aca cta cca cag tgt cag gca gcg aac aag acc     1077
Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr
            230                 235                 240

tgc ccc acc aat tac atg tgg aat aat cac atc tgc aga tgc ctg gct     1125
Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala
        245                 250                 255

cag gaa gat ttt atg ttt tcc tcg gat gct gga gat gac tca aca gat     1173
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Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr
275                 280                 285                 290

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Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn
    340                 345                 350

cct gga aaa tgt gcc tgt gaa tgt aca gaa agt cca cag aaa tgc ttg     1461
Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu
355                 360                 365                 370

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cca tgt acg aac cgc cag aag gct tgt gag cca gga ttt tca tat agt     1557
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Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
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Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
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Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
                85                  90                  95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
            100                 105                 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
        115                 120                 125


Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
    130                 135                 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
145                 150                 155                 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
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Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
            180                 185                 190

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
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Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
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Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225                 230                 235                 240

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                245                 250                 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
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Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
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Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
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Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
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tgaagttttg aggtttcaaa ctttccttct ggagaatgcc ttttgaaaca attttctcta    360

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                                                          1

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    20                  25                  30

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Ser Thr Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Ser Leu
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Cys Arg Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg Ser Ala
            70                  75                  80

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Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile Glu Thr
        85                  90                  95

cta aaa gtt ata gat gaa gaa tgg caa aga act cag tgc agc cct aga      752
Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro Arg
    100                 105                 110

gaa acg tgc gtg gag gtg gcc agt gag ctg ggg aag agt acc aac aca      800
Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr Asn Thr
115                 120                 125                 130

ttc ttc aag ccc cct tgt gtg aac gtg ttc cga tgt ggt ggc tgt tgc      848
Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys Cys
                135                 140                 145

aat gaa gag agc ctt atc tgt atg aac acc agc acc tcg tac att tcc      896
Asn Glu Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr Ile Ser
            150                 155                 160

aaa cag ctc ttt gag ata tca gtg cct ttg aca tca gta cct gaa tta      944
Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu Leu
        165                 170                 175

gtg cct gtt aaa gtt gcc aat cat aca ggt tgt aag tgc ttg cca aca      992
Val Pro Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu Pro Thr
    180                 185                 190

gcc ccc cgc cat cca tac tca att atc aga aga tcc atc cag atc cct     1040
Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Ile Pro
195                 200                 205                 210

gaa gaa gat cgc tgt tcc cat tcc aag aaa ctc tgt cct att gac atg     1088
Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile Asp Met
                215                 220                 225

cta tgg gat agc aac aaa tgt aaa tgt gtt ttg cag gag gaa aat cca     1136
Leu Trp Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu Asn Pro
            230                 235                 240

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Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala Leu Cys
        245                 250                 255

ggg cca cac atg atg ttt gac gaa gat cgt tgc gag tgt gtc tgt aaa     1232
Gly Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val Cys Lys
    260                 265                 270

aca cca tgt ccc aaa gat cta atc cag cac ccc aaa aac tgc agt tgc     1280
Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys Ser Cys
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            310                 315                 320

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Arg Pro Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys Arg Phe
        325                 330                 335

cca aag gag aaa agg gct gcc cag ggg ccc cac agc cga aag aat cct     1472
Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys Asn Pro
    340                 345                 350

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        35                  40                  45

Ser Leu Glu Glu Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu
    50                  55                  60

Trp Arg Cys Arg Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg
65                  70                  75                  80
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                85                  90                  95

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            100                 105                 110

Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr
        115                 120                 125

Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly
    130                 135                 140

Cys Cys Asn Glu Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr
145                 150                 155                 160

Ile Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro
                165                 170                 175

Glu Leu Val Pro Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu
            180                 185                 190

Pro Thr Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln
        195                 200                 205

Ile Pro Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile
    210                 215                 220

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225                 230                 235                 240

Asn Pro Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala
                245                 250                 255

Leu Cys Gly Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val
            260                 265                 270

Cys Lys Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys
        275                 280                 285

Ser Cys Phe Glu Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His
    290                 295                 300

Lys Leu Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe
305                 310                 315                 320

His Thr Arg Pro Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys
                325                 330                 335
```

```
Arg Phe Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys
            340                 345                 350

Asn Pro
```

<210> 5
<211> 232
<212> PRT
<213> Homo sapiens

<400> 5

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                  10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
    130                 135                 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145                 150                 155                 160

Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp
                165                 170                 175

Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys
            180                 185                 190
```

```
His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn
        195                 200                 205

Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr
    210                 215                 220

Cys Arg Cys Asp Lys Pro Arg Arg
225                 230
```

<210> 6
<211> 576
<212> DNA
<213> Homo sapiens

<400> 6

```
atgaactttc tgctgtcttg ggtgcattgg agccttgcct tgctgctcta cctccaccat     60

gccaagtggt cccaggctgc acccatggca gaaggaggag ggcagaatca tcacgaagtg    120

gtgaagttca tggatgtcta tcagcgcagc tactgccatc caatcgagac cctggtggac    180

atcttccagg agtaccctga tgagatcgag tacatcttca agccatcctg tgtgcccctg    240

atgcgatgcg ggggctgctg caatgacgag ggcctggagt gtgtgcccac tgaggagtcc    300

aacatcacca tgcagattat gcggatcaaa cctcaccaag gccagcacat aggagagatg    360

agcttcctac agcacaacaa atgtgaatgc agaccaaaga aagatagagc aagacaagaa    420

aatccctgtg ggccttgctc agagcggaga aagcatttgt ttgtacaaga tccgcagacg    480

tgtaaatgtt cctgcaaaaa cacagactcg cgttgcaagg cgaggcagct tgagttaaac    540

gaacgtactt gcagatgtga caagccgagg cggtga                               576
```

<210> 7
<211> 191
<212> PRT
<213> Homo sapiens

<400> 7

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
```

65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130 135 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145 150 155 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165 170 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185 190

<210> 8
<211> 188
<212> PRT
<213> Homo sapiens

<400> 8

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
20 25 30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65 70 75 80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
85 90 95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
100 105 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
115 120 125

Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
130 135 140

Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Arg Arg
145 150 155 160

Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
165 170 175

Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
180 185

<210> 9
<211> 106
<212> DNA
<213> Homo sapiens

<400> 9
gtttttttat tttccagaaa atcagttcga ggaaagggaa aggggcaaaa acgaaagcgc 60

aagaaatccc ggtataagtc ctggagcgtg tacgttggtg cccgct 106

<210> 10
<211> 215
<212> PRT
<213> Homo sapiens

<400> 10

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

```
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
    130                 135                 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145                 150                 155                 160

Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His
                165                 170                 175

Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr
            180                 185                 190

Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys
        195                 200                 205

Arg Cys Asp Lys Pro Arg Arg
    210                 215
```

<210> 11
<211> 166
<212> DNA
<213> Homo sapiens

<400> 11

```
cttttgcctt tttgcagtcc ctgtgggcct tgctcagagc ggagaaagca tttgtttgta     60

caagatccgc agacgtgtaa atgttcctgc aaaaacacag actcgcgttg caaggcgagg    120

cagcttgagt taaacgaacg tacttgcagg ttggttccca gaggca                   166
```

<210> 12
<211> 161
<212> PRT
<213> Homo sapiens

<400> 12

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                   10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
```

35 40 45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130 135 140

Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg
145 150 155 160

Arg

<210> 13
<211> 76
<212> DNA
<213> Homo sapiens

<400> 13
ttttccattt ccctcagatg tgacaagccg aggcggtgag ccgggcagga ggaaggagcc 60

tccctcaggg tttcgg 76

<210> 14
<211> 181
<212> PRT
<213> Homo sapiens

<400> 14

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45

```
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
    130                 135                 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145                 150                 155                 160

Lys Ser Trp Ser Val Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys
                165                 170                 175

Asp Lys Pro Arg Arg
            180
```

<210> 15
<211> 221
<212> PRT
<213> Homo sapiens

<400> 15

```
Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1               5                   10                  15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
            20                  25                  30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
        35                  40                  45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
    50                  55                  60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65                  70                  75                  80
```

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
85 90 95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
100 105 110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115 120 125

Glu Cys Arg His Ser Pro Gly Arg Gln Ser Pro Asp Met Pro Gly Asp
130 135 140

Phe Arg Ala Asp Ala Pro Ser Phe Leu Pro Pro Arg Arg Ser Leu Pro
145 150 155 160

Met Leu Phe Arg Met Glu Trp Gly Cys Ala Leu Thr Gly Ser Gln Ser
165 170 175

Ala Val Trp Pro Ser Ser Pro Val Pro Glu Glu Ile Pro Arg Met His
180 185 190

Pro Gly Arg Asn Gly Lys Lys Gln Gln Arg Lys Pro Leu Arg Glu Lys
195 200 205

Met Lys Pro Glu Arg Cys Gly Asp Ala Val Pro Arg Arg
210 215 220

<210> 16
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic primer

<400> 16
acattggtgt gcacctccaa gc 22

<210> 17
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic primer

<400> 17
aataatggaa tgaacttgtc tgtaaac 27

<210> 18
<211> 24
<212> DNA

<213> Artificial sequence

<220>
<223> Synthetic primer

<400> 18
aaatcagttc gaggaaaggg aaag 24

<210> 19
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic primer

<400> 19
ccctgtgggc cttgctcaga g 21

<210> 20
<211> 35
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic primer

<400> 20
ccatgctcga gagtctttcc tggtgagaga tctgg 35

<210> 21
<211> 682
<212> PRT
<213> Homo sapiens

<400> 21

Met Val Ser Trp Gly Arg Phe Ile Cys Leu Val Val Val Thr Met Ala
1 5 10 15

Thr Leu Ser Leu Ala Arg Pro Ser Phe Ser Leu Val Glu Asp Thr Thr
20 25 30

Leu Glu Pro Glu Asp Ala Ile Ser Ser Gly Asp Asp Glu Asp Asp Thr
35 40 45

Asp Gly Ala Glu Asp Phe Val Ser Glu Asn Ser Asn Asn Lys Arg Ala
50 55 60

Pro Tyr Trp Thr Asn Thr Glu Lys Met Glu Lys Arg Leu His Ala Val
65 70 75 80

Pro Ala Ala Asn Thr Val Lys Phe Arg Cys Pro Ala Gly Gly Asn Pro
85 90 95

```
Met Pro Thr Met Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Gln Glu
            100                 105                 110

His Arg Ile Gly Gly Tyr Lys Val Arg Asn Gln His Trp Ser Leu Ile
        115                 120                 125

Met Glu Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Val Val
    130                 135                 140

Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr His Leu Asp Val Val
145                 150                 155                 160

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
                165                 170                 175

Ala Ser Thr Val Val Gly Gly Asp Val Glu Phe Val Cys Lys Val Tyr
            180                 185                 190

Ser Asp Ala Gln Pro His Ile Gln Trp Ile Lys His Val Glu Lys Asn
        195                 200                 205

Gly Ser Lys Tyr Gly Pro Asp Gly Leu Pro Tyr Leu Lys Val Leu Lys
    210                 215                 220

His Ser Gly Ile Asn Ser Ser Asn Ala Glu Val Leu Ala Leu Phe Asn
225                 230                 235                 240

Val Thr Glu Ala Asp Ala Gly Glu Tyr Ile Cys Lys Val Ser Asn Tyr
                245                 250                 255

Ile Gly Gln Ala Asn Gln Ser Ala Trp Leu Thr Val Leu Pro Lys Gln
            260                 265                 270

Gln Ala Pro Gly Arg Glu Lys Glu Ile Thr Ala Ser Pro Asp Tyr Leu
        275                 280                 285

Glu Ile Ala Ile Tyr Cys Ile Gly Val Phe Leu Ile Ala Cys Met Val
    290                 295                 300

Val Thr Val Ile Leu Cys Arg Met Lys Asn Thr Thr Lys Lys Pro Asp
305                 310                 315                 320

Phe Ser Ser Gln Pro Ala Val His Lys Leu Thr Lys Arg Ile Pro Leu
                325                 330                 335

Arg Arg Gln Val Ser Ala Glu Ser Ser Ser Ser Met Asn Ser Asn Thr
            340                 345                 350
```

```
Pro Leu Val Arg Ile Thr Thr Arg Leu Ser Ser Thr Ala Asp Thr Pro
        355                 360                 365

Met Leu Ala Gly Val Ser Glu Tyr Glu Leu Pro Glu Asp Pro Lys Trp
    370                 375                 380

Glu Phe Pro Arg Asp Lys Leu Thr Leu Gly Lys Pro Leu Gly Glu Gly
385                 390                 395                 400

Cys Phe Gly Gln Val Val Met Ala Glu Ala Val Gly Ile Asp Lys Asp
                405                 410                 415

Lys Pro Lys Glu Ala Val Thr Val Ala Val Lys Met Leu Lys Asp Asp
            420                 425                 430

Ala Thr Glu Lys Asp Leu Ser Asp Leu Val Ser Glu Met Glu Met Met
        435                 440                 445

Lys Met Ile Gly Lys His Lys Asn Ile Ile Asn Leu Leu Gly Ala Cys
    450                 455                 460

Thr Gln Asp Gly Pro Leu Tyr Val Ile Val Glu Tyr Ala Ser Lys Gly
465                 470                 475                 480

Asn Leu Arg Glu Tyr Leu Arg Ala Arg Arg Pro Pro Gly Met Glu Tyr
                485                 490                 495

Ser Tyr Asp Ile Asn Arg Val Pro Glu Glu Gln Met Thr Phe Lys Asp
            500                 505                 510

Leu Val Ser Cys Thr Tyr Gln Leu Ala Arg Gly Met Glu Tyr Leu Ala
        515                 520                 525

Ser Gln Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Val Leu Val
    530                 535                 540

Thr Glu Asn Asn Val Met Lys Ile Ala Asp Phe Gly Leu Ala Arg Asp
545                 550                 555                 560

Ile Asn Asn Ile Asp Tyr Tyr Lys Lys Thr Thr Asn Gly Arg Leu Pro
                565                 570                 575

Val Lys Trp Met Ala Pro Glu Ala Leu Phe Asp Arg Val Tyr Thr His
            580                 585                 590

Gln Ser Asp Val Trp Ser Phe Gly Val Leu Met Trp Glu Ile Phe Thr
        595                 600                 605
```

```
Leu Gly Gly Ser Pro Tyr Pro Gly Ile Pro Val Glu Glu Leu Phe Lys
    610                 615                 620

Leu Leu Lys Glu Gly His Arg Met Asp Lys Pro Ala Asn Cys Thr Asn
625                 630                 635                 640

Glu Leu Tyr Met Met Met Arg Asp Cys Trp His Ala Val Pro Ser Gln
                645                 650                 655

Arg Pro Thr Phe Lys Gln Leu Val Glu Asp Leu Asp Arg Ile Pro Pro
            660                 665                 670

Asn Pro Ser Leu Met Ser Ile Phe Arg Lys
        675                 680
```

<210> 22
<211> 208
<212> PRT
<213> Homo sapiens

<400> 22

```
Met Lys Leu Leu Pro Ser Val Val Leu Lys Leu Phe Leu Ala Ala Val
1               5                   10                  15

Leu Ser Ala Leu Val Thr Gly Glu Ser Leu Glu Arg Leu Arg Arg Gly
            20                  25                  30

Leu Ala Ala Gly Thr Ser Asn Pro Asp Pro Pro Thr Val Ser Thr Asp
        35                  40                  45

Gln Leu Leu Pro Leu Gly Gly Gly Arg Asp Arg Lys Val Arg Asp Leu
    50                  55                  60

Gln Glu Ala Asp Leu Asp Leu Leu Arg Val Thr Leu Ser Ser Lys Pro
65                  70                  75                  80

Gln Ala Leu Ala Thr Pro Asn Lys Glu Glu His Gly Lys Arg Lys Lys
                85                  90                  95

Lys Gly Lys Gly Leu Gly Lys Lys Arg Asp Pro Cys Leu Arg Lys Tyr
            100                 105                 110

Lys Asp Phe Cys Ile His Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg
        115                 120                 125

Ala Pro Ser Cys Ile Cys His Pro Gly Tyr His Gly Glu Arg Cys His
    130                 135                 140
```

```
Gly Leu Ser Leu Pro Val Glu Asn Arg Leu Tyr Thr Tyr Asp His Thr
145                 150                 155                 160

Thr Ile Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu
                165                 170                 175

Val Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg Arg Gly Gly Tyr
            180                 185                 190

Asp Val Glu Asn Glu Glu Lys Val Lys Leu Gly Met Thr Asn Ser His
        195                 200                 205
```

<210> 23
<211> 234
<212> PRT
<213> Homo sapiens

<400> 23

```
Met Lys Ile Cys Ser Leu Thr Leu Leu Ser Phe Leu Leu Leu Ala Ala
1               5                   10                  15

Gln Val Leu Leu Val Glu Gly Lys Lys Lys Val Lys Asn Gly Leu His
            20                  25                  30

Ser Lys Val Val Ser Glu Gln Lys Asp Thr Leu Gly Asn Thr Gln Ile
        35                  40                  45

Lys Gln Lys Ser Arg Pro Gly Asn Lys Gly Lys Phe Val Thr Lys Asp
    50                  55                  60

Gln Ala Asn Cys Arg Trp Ala Ala Thr Glu Gln Glu Glu Gly Ile Ser
65                  70                  75                  80

Leu Lys Val Glu Cys Thr Gln Leu Asp His Glu Phe Ser Cys Val Phe
                85                  90                  95

Ala Gly Asn Pro Thr Ser Cys Leu Lys Leu Lys Asp Glu Arg Val Tyr
            100                 105                 110

Trp Lys Gln Val Ala Arg Asn Leu Arg Ser Gln Lys Asp Ile Cys Arg
        115                 120                 125

Tyr Ser Lys Thr Ala Val Lys Thr Arg Val Cys Arg Lys Asp Phe Pro
    130                 135                 140

Glu Ser Ser Leu Lys Leu Val Ser Ser Thr Leu Phe Gly Asn Thr Lys
145                 150                 155                 160
```

```
Pro Arg Lys Glu Lys Thr Glu Met Ser Pro Arg Glu His Ile Lys Gly
                165                 170                 175

Lys Glu Thr Thr Pro Ser Ser Leu Ala Val Thr Gln Thr Met Ala Thr
            180                 185                 190

Lys Ala Pro Glu Cys Val Glu Asp Pro Asp Met Ala Asn Gln Arg Lys
        195                 200                 205

Thr Ala Leu Glu Phe Cys Gly Glu Thr Trp Ser Ser Leu Cys Thr Phe
    210                 215                 220

Phe Leu Ser Ile Val Gln Asp Thr Ser Cys
225                 230
```

<210> 24
<211> 452
<212> PRT
<213> Homo sapiens

<400> 24

```
Met Asp Ser Gly Arg Arg Leu Gly Pro Glu Lys Trp Ile Arg Arg Leu
1               5                   10                  15

Arg Arg Met Glu Ser Glu Ser Glu Ser Gly Ala Ala Ala Asp Thr Pro
            20                  25                  30

Pro Leu Glu Thr Leu Ser Phe His Gly Asp Glu Glu Ile Ile Glu Val
        35                  40                  45

Val Glu Leu Asp Pro Gly Pro Pro Asp Pro Asp Asp Leu Ala Gln Glu
    50                  55                  60

Met Glu Asp Val Asp Phe Glu Glu Glu Glu Glu Glu Glu Gly Asn Glu
65                  70                  75                  80

Glu Gly Trp Val Leu Glu Pro Gln Glu Gly Val Val Gly Ser Met Glu
                85                  90                  95

Gly Pro Asp Asp Ser Glu Val Thr Phe Ala Leu His Ser Ala Ser Val
            100                 105                 110

Phe Cys Val Ser Leu Asp Pro Lys Thr Asn Thr Leu Ala Val Thr Gly
        115                 120                 125

Gly Glu Asp Asp Lys Ala Phe Val Trp Arg Leu Ser Asp Gly Glu Leu
    130                 135                 140
```

```
Leu Phe Glu Cys Ala Gly His Lys Asp Ser Val Thr Cys Ala Gly Phe
145                 150                 155                 160

Ser His Asp Ser Thr Leu Val Ala Thr Gly Asp Met Ser Gly Leu Leu
                165                 170                 175

Lys Val Trp Gln Val Asp Thr Lys Glu Glu Val Trp Ser Phe Glu Ala
            180                 185                 190

Gly Asp Leu Glu Trp Met Glu Trp His Pro Arg Ala Pro Val Leu Leu
        195                 200                 205

Ala Gly Thr Ala Asp Gly Asn Thr Trp Met Trp Lys Val Pro Asn Gly
    210                 215                 220

Asp Cys Lys Thr Phe Gln Gly Pro Asn Cys Pro Ala Thr Cys Gly Arg
225                 230                 235                 240

Val Leu Pro Asp Gly Lys Arg Ala Val Val Gly Tyr Glu Asp Gly Thr
                245                 250                 255

Ile Arg Ile Trp Asp Leu Lys Gln Gly Ser Pro Ile His Val Leu Lys
            260                 265                 270

Gly Thr Glu Gly His Gln Gly Pro Leu Thr Cys Val Ala Ala Asn Gln
        275                 280                 285

Asp Gly Ser Leu Ile Leu Thr Gly Ser Val Asp Cys Gln Ala Lys Leu
    290                 295                 300

Val Ser Ala Thr Thr Gly Lys Val Val Gly Val Phe Arg Pro Glu Thr
305                 310                 315                 320

Val Ala Ser Gln Pro Ser Leu Gly Glu Gly Glu Glu Ser Glu Ser Asn
                325                 330                 335

Ser Val Glu Ser Leu Gly Phe Cys Ser Val Met Pro Leu Ala Ala Val
            340                 345                 350

Gly Tyr Leu Asp Gly Thr Leu Ala Ile Tyr Asp Leu Ala Thr Gln Thr
        355                 360                 365

Leu Arg His Gln Cys Gln His Gln Ser Gly Ile Val Gln Leu Leu Trp
    370                 375                 380

Glu Ala Gly Thr Ala Val Val Tyr Thr Cys Ser Leu Asp Gly Ile Val
385                 390                 395                 400
```

```
Arg Leu Trp Asp Ala Arg Thr Gly Arg Leu Leu Thr Asp Tyr Arg Gly
                405                 410                 415

His Thr Ala Glu Ile Leu Asp Phe Ala Leu Ser Lys Asp Ala Ser Leu
            420                 425                 430

Val Val Thr Thr Ser Gly Asp His Lys Ala Lys Val Phe Cys Val Gln
        435                 440                 445

Arg Pro Asp Arg
    450
```

<210> 25
<211> 4563
<212> PRT
<213> Homo sapiens

<220> misc_feature
<222> (32)..(126)
<223> heparin binding domain

<220> misc_feature
<222> (3161)..(3236)
<223> heparin binding domain

<400> 25

```
Met Asp Pro Pro Arg Pro Ala Leu Leu Ala Leu Leu Ala Leu Pro Ala
1               5                   10                  15

Leu Leu Leu Leu Leu Leu Ala Gly Ala Arg Ala Glu Glu Glu Met Leu
            20                  25                  30

Glu Asn Val Ser Leu Val Cys Pro Lys Asp Ala Thr Arg Phe Lys His
        35                  40                  45

Leu Arg Lys Tyr Thr Tyr Asn Tyr Glu Ala Glu Ser Ser Ser Gly Val
    50                  55                  60

Pro Gly Thr Ala Asp Ser Arg Ser Ala Thr Arg Ile Asn Cys Lys Val
65                  70                  75                  80

Glu Leu Glu Val Pro Gln Leu Cys Ser Phe Ile Leu Lys Thr Ser Gln
                85                  90                  95

Cys Thr Leu Lys Glu Val Tyr Gly Phe Asn Pro Glu Gly Lys Ala Leu
            100                 105                 110

Leu Lys Lys Thr Lys Asn Ser Glu Glu Phe Ala Ala Ala Met Ser Arg
        115                 120                 125
```

```
Tyr Glu Leu Lys Leu Ala Ile Pro Glu Gly Lys Gln Val Phe Leu Tyr
    130                 135                 140

Pro Glu Lys Asp Glu Pro Thr Tyr Ile Leu Asn Ile Lys Arg Gly Ile
145                 150                 155                 160

Ile Ser Ala Leu Leu Val Pro Pro Glu Thr Glu Glu Ala Lys Gln Val
                165                 170                 175

Leu Phe Leu Asp Thr Val Tyr Gly Asn Cys Ser Thr His Phe Thr Val
            180                 185                 190

Lys Thr Arg Lys Gly Asn Val Ala Thr Glu Ile Ser Thr Glu Arg Asp
        195                 200                 205

Leu Gly Gln Cys Asp Arg Phe Lys Pro Ile Arg Thr Gly Ile Ser Pro
    210                 215                 220

Leu Ala Leu Ile Lys Gly Met Thr Arg Pro Leu Ser Thr Leu Ile Ser
225                 230                 235                 240

Ser Ser Gln Ser Cys Gln Tyr Thr Leu Asp Ala Lys Arg Lys His Val
                245                 250                 255

Ala Glu Ala Ile Cys Lys Glu Gln His Leu Phe Leu Pro Phe Ser Tyr
            260                 265                 270

Lys Asn Lys Tyr Gly Met Val Ala Gln Val Thr Gln Thr Leu Lys Leu
        275                 280                 285

Glu Asp Thr Pro Lys Ile Asn Ser Arg Phe Phe Gly Glu Gly Thr Lys
    290                 295                 300

Lys Met Gly Leu Ala Phe Glu Ser Thr Lys Ser Thr Ser Pro Pro Lys
305                 310                 315                 320

Gln Ala Glu Ala Val Leu Lys Thr Leu Gln Glu Leu Lys Lys Leu Thr
                325                 330                 335

Ile Ser Glu Gln Asn Ile Gln Arg Ala Asn Leu Phe Asn Lys Leu Val
            340                 345                 350

Thr Glu Leu Arg Gly Leu Ser Asp Glu Ala Val Thr Ser Leu Leu Pro
        355                 360                 365

Gln Leu Ile Glu Val Ser Ser Pro Ile Thr Leu Gln Ala Leu Val Gln
    370                 375                 380
```

```
Cys Gly Gln Pro Gln Cys Ser Thr His Ile Leu Gln Trp Leu Lys Arg
385                 390                 395                 400

Val His Ala Asn Pro Leu Leu Ile Asp Val Val Thr Tyr Leu Val Ala
                405                 410                 415

Leu Ile Pro Glu Pro Ser Ala Gln Gln Leu Arg Glu Ile Phe Asn Met
            420                 425                 430

Ala Arg Asp Gln Arg Ser Arg Ala Thr Leu Tyr Ala Leu Ser His Ala
        435                 440                 445

Val Asn Asn Tyr His Lys Thr Asn Pro Thr Gly Thr Gln Glu Leu Leu
    450                 455                 460

Asp Ile Ala Asn Tyr Leu Met Glu Gln Ile Gln Asp Asp Cys Thr Gly
465                 470                 475                 480

Asp Glu Asp Tyr Thr Tyr Leu Ile Leu Arg Val Ile Gly Asn Met Gly
                485                 490                 495

Gln Thr Met Glu Gln Leu Thr Pro Glu Leu Lys Ser Ser Ile Leu Lys
            500                 505                 510

Cys Val Gln Ser Thr Lys Pro Ser Leu Met Ile Gln Lys Ala Ala Ile
        515                 520                 525

Gln Ala Leu Arg Lys Met Glu Pro Lys Asp Lys Asp Gln Glu Val Leu
    530                 535                 540

Leu Gln Thr Phe Leu Asp Asp Ala Ser Pro Gly Asp Lys Arg Leu Ala
545                 550                 555                 560

Ala Tyr Leu Met Leu Met Arg Ser Pro Ser Gln Ala Asp Ile Asn Lys
                565                 570                 575

Ile Val Gln Ile Leu Pro Trp Glu Gln Asn Glu Gln Val Lys Asn Phe
            580                 585                 590

Val Ala Ser His Ile Ala Asn Ile Leu Asn Ser Glu Glu Leu Asp Ile
        595                 600                 605

Gln Asp Leu Lys Lys Leu Val Lys Glu Val Leu Lys Glu Ser Gln Leu
    610                 615                 620

Pro Thr Val Met Asp Phe Arg Lys Phe Ser Arg Asn Tyr Gln Leu Tyr
625                 630                 635                 640
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Lys Ser Val Ser Leu Pro Ser Leu Asp Pro Ala Ser Ala Lys Ile Glu
                645                 650                 655

Gly Asn Leu Ile Phe Asp Pro Asn Asn Tyr Leu Pro Lys Glu Ser Met
            660                 665                 670

Leu Lys Thr Thr Leu Thr Ala Phe Gly Phe Ala Ser Ala Asp Leu Ile
        675                 680                 685

Glu Ile Gly Leu Glu Gly Lys Gly Phe Glu Pro Thr Leu Glu Ala Leu
    690                 695                 700

Phe Gly Lys Gln Gly Phe Phe Pro Asp Ser Val Asn Lys Ala Leu Tyr
705                 710                 715                 720

Trp Val Asn Gly Gln Val Pro Asp Gly Val Ser Lys Val Leu Val Asp
                725                 730                 735

His Phe Gly Tyr Thr Lys Asp Asp Lys His Glu Gln Asp Met Val Asn
            740                 745                 750

Gly Ile Met Leu Ser Val Glu Lys Leu Ile Lys Asp Leu Lys Ser Lys
        755                 760                 765

Glu Val Pro Glu Ala Arg Ala Tyr Leu Arg Ile Leu Gly Glu Glu Leu
    770                 775                 780

Gly Phe Ala Ser Leu His Asp Leu Gln Leu Leu Gly Lys Leu Leu Leu
785                 790                 795                 800

Met Gly Ala Arg Thr Leu Gln Gly Ile Pro Gln Met Ile Gly Glu Val
                805                 810                 815

Ile Arg Lys Gly Ser Lys Asn Asp Phe Phe Leu His Tyr Ile Phe Met
            820                 825                 830

Glu Asn Ala Phe Glu Leu Pro Thr Gly Ala Gly Leu Gln Leu Gln Ile
        835                 840                 845

Ser Ser Ser Gly Val Ile Ala Pro Gly Ala Lys Ala Gly Val Lys Leu
    850                 855                 860

Glu Val Ala Asn Met Gln Ala Glu Leu Val Ala Lys Pro Ser Val Ser
865                 870                 875                 880

Val Glu Phe Val Thr Asn Met Gly Ile Ile Ile Pro Asp Phe Ala Arg
                885                 890                 895
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Ser Gly Val Gln Met Asn Thr Asn Phe Phe His Glu Ser Gly Leu Glu
            900                 905                 910

Ala His Val Ala Leu Lys Ala Gly Lys Leu Lys Phe Ile Ile Pro Ser
        915                 920                 925

Pro Lys Arg Pro Val Lys Leu Leu Ser Gly Gly Asn Thr Leu His Leu
    930                 935                 940

Val Ser Thr Thr Lys Thr Glu Val Ile Pro Pro Leu Ile Glu Asn Arg
945                 950                 955                 960

Gln Ser Trp Ser Val Cys Lys Gln Val Phe Pro Gly Leu Asn Tyr Cys
                965                 970                 975

Thr Ser Gly Ala Tyr Ser Asn Ala Ser Ser Thr Asp Ser Ala Ser Tyr
            980                 985                 990

Tyr Pro Leu Thr Gly Asp Thr Arg  Leu Glu Leu Glu Leu  Arg Pro Thr
        995                 1000                1005

Gly Glu  Ile Glu Gln Tyr Ser  Val Ser Ala Thr Tyr  Glu Leu Gln
    1010                1015                1020

Arg Glu  Asp Arg Ala Leu Val  Asp Thr Leu Lys Phe  Val Thr Gln
    1025                1030                1035

Ala Glu  Gly Ala Lys Gln Thr  Glu Ala Thr Met Thr  Phe Lys Tyr
    1040                1045                1050

Asn Arg  Gln Ser Met Thr Leu  Ser Ser Glu Val Gln  Ile Pro Asp
    1055                1060                1065

Phe Asp  Val Asp Leu Gly Thr  Ile Leu Arg Val Asn  Asp Glu Ser
    1070                1075                1080

Thr Glu  Gly Lys Thr Ser Tyr  Arg Leu Thr Leu Asp  Ile Gln Asn
    1085                1090                1095

Lys Lys  Ile Thr Glu Val Ala  Leu Met Gly His Leu  Ser Cys Asp
    1100                1105                1110

Thr Lys  Glu Glu Arg Lys Ile  Lys Gly Val Ile Ser  Ile Pro Arg
    1115                1120                1125

Leu Gln  Ala Glu Ala Arg Ser  Glu Ile Leu Ala His  Trp Ser Pro
    1130                1135                1140
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Ala Lys  Leu Leu Leu Gln Met  Asp Ser Ser Ala Thr  Ala Tyr Gly
    1145                1150                1155

Ser Thr  Val Ser Lys Arg Val  Ala Trp His Tyr Asp  Glu Glu Lys
    1160                1165                1170

Ile Glu  Phe Glu Trp Asn Thr  Gly Thr Asn Val Asp  Thr Lys Lys
    1175                1180                1185

Met Thr  Ser Asn Phe Pro Val  Asp Leu Ser Asp Tyr  Pro Lys Ser
    1190                1195                1200

Leu His  Met Tyr Ala Asn Arg  Leu Leu Asp His Arg  Val Pro Gln
    1205                1210                1215

Thr Asp  Met Thr Phe Arg His  Val Gly Ser Lys Leu  Ile Val Ala
    1220                1225                1230

Met Ser  Ser Trp Leu Gln Lys  Ala Ser Gly Ser Leu  Pro Tyr Thr
    1235                1240                1245

Gln Thr  Leu Gln Asp His Leu  Asn Ser Leu Lys Glu  Phe Asn Leu
    1250                1255                1260

Gln Asn  Met Gly Leu Pro Asp  Phe His Ile Pro Glu  Asn Leu Phe
    1265                1270                1275

Leu Lys  Ser Asp Gly Arg Val  Lys Tyr Thr Leu Asn  Lys Asn Ser
    1280                1285                1290

Leu Lys  Ile Glu Ile Pro Leu  Pro Phe Gly Gly Lys  Ser Ser Arg
    1295                1300                1305

Asp Leu  Lys Met Leu Glu Thr  Val Arg Thr Pro Ala  Leu His Phe
    1310                1315                1320

Lys Ser  Val Gly Phe His Leu  Pro Ser Arg Glu Phe  Gln Val Pro
    1325                1330                1335

Thr Phe  Thr Ile Pro Lys Leu  Tyr Gln Leu Gln Val  Pro Leu Leu
    1340                1345                1350

Gly Val  Leu Asp Leu Ser Thr  Asn Val Tyr Ser Asn  Leu Tyr Asn
    1355                1360                1365

Trp Ser  Ala Ser Tyr Ser Gly  Gly Asn Thr Ser Thr  Asp His Phe
    1370                1375                1380
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Ser Leu  Arg Ala Arg Tyr His  Met Lys Ala Asp Ser  Val Val Asp
    1385                 1390                 1395

Leu Leu  Ser Tyr Asn Val Gln  Gly Ser Gly Glu Thr  Thr Tyr Asp
    1400                 1405                 1410

His Lys  Asn Thr Phe Thr Leu  Ser Cys Asp Gly Ser  Leu Arg His
    1415                 1420                 1425

Lys Phe  Leu Asp Ser Asn Ile  Lys Phe Ser His Val  Glu Lys Leu
    1430                 1435                 1440

Gly Asn  Asn Pro Val Ser Lys  Gly Leu Leu Ile Phe  Asp Ala Ser
    1445                 1450                 1455

Ser Ser  Trp Gly Pro Gln Met  Ser Ala Ser Val His  Leu Asp Ser
    1460                 1465                 1470

Lys Lys  Lys Gln His Leu Phe  Val Lys Glu Val Lys  Ile Asp Gly
    1475                 1480                 1485

Gln Phe  Arg Val Ser Ser Phe  Tyr Ala Lys Gly Thr  Tyr Gly Leu
    1490                 1495                 1500

Ser Cys  Gln Arg Asp Pro Asn  Thr Gly Arg Leu Asn  Gly Glu Ser
    1505                 1510                 1515

Asn Leu  Arg Phe Asn Ser Ser  Tyr Leu Gln Gly Thr  Asn Gln Ile
    1520                 1525                 1530

Thr Gly  Arg Tyr Glu Asp Gly  Thr Leu Ser Leu Thr  Ser Thr Ser
    1535                 1540                 1545

Asp Leu  Gln Ser Gly Ile Ile  Lys Asn Thr Ala Ser  Leu Lys Tyr
    1550                 1555                 1560

Glu Asn  Tyr Glu Leu Thr Leu  Lys Ser Asp Thr Asn  Gly Lys Tyr
    1565                 1570                 1575

Lys Asn  Phe Ala Thr Ser Asn  Lys Met Asp Met Thr  Phe Ser Lys
    1580                 1585                 1590

Gln Asn  Ala Leu Leu Arg Ser  Glu Tyr Gln Ala Asp  Tyr Glu Ser
    1595                 1600                 1605

Leu Arg  Phe Phe Ser Leu Leu  Ser Gly Ser Leu Asn  Ser His Gly
    1610                 1615                 1620
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Leu Glu Leu Asn Ala Asp Ile Leu Gly Thr Asp Lys Ile Asn Ser
1625 1630 1635

Gly Ala His Lys Ala Thr Leu Arg Ile Gly Gln Asp Gly Ile Ser
1640 1645 1650

Thr Ser Ala Thr Thr Asn Leu Lys Cys Ser Leu Leu Val Leu Glu
1655 1660 1665

Asn Glu Leu Asn Ala Glu Leu Gly Leu Ser Gly Ala Ser Met Lys
1670 1675 1680

Leu Thr Thr Asn Gly Arg Phe Arg Glu His Asn Ala Lys Phe Ser
1685 1690 1695

Leu Asp Gly Lys Ala Ala Leu Thr Glu Leu Ser Leu Gly Ser Ala
1700 1705 1710

Tyr Gln Ala Met Ile Leu Gly Val Asp Ser Lys Asn Ile Phe Asn
1715 1720 1725

Phe Lys Val Ser Gln Glu Gly Leu Lys Leu Ser Asn Asp Met Met
1730 1735 1740

Gly Ser Tyr Ala Glu Met Lys Phe Asp His Thr Asn Ser Leu Asn
1745 1750 1755

Ile Ala Gly Leu Ser Leu Asp Phe Ser Ser Lys Leu Asp Asn Ile
1760 1765 1770

Tyr Ser Ser Asp Lys Phe Tyr Lys Gln Thr Val Asn Leu Gln Leu
1775 1780 1785

Gln Pro Tyr Ser Leu Val Thr Thr Leu Asn Ser Asp Leu Lys Tyr
1790 1795 1800

Asn Ala Leu Asp Leu Thr Asn Asn Gly Lys Leu Arg Leu Glu Pro
1805 1810 1815

Leu Lys Leu His Val Ala Gly Asn Leu Lys Gly Ala Tyr Gln Asn
1820 1825 1830

Asn Glu Ile Lys His Ile Tyr Ala Ile Ser Ser Ala Ala Leu Ser
1835 1840 1845

Ala Ser Tyr Lys Ala Asp Thr Val Ala Lys Val Gln Gly Val Glu
1850 1855 1860

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Phe Ser  His Arg Leu Asn Thr  Asp Ile Ala Gly Leu  Ala Ser Ala
    1865                 1870                 1875

Ile Asp  Met Ser Thr Asn Tyr  Asn Ser Asp Ser Leu  His Phe Ser
    1880                 1885                 1890

Asn Val  Phe Arg Ser Val Met  Ala Pro Phe Thr Met  Thr Ile Asp
    1895                 1900                 1905

Ala His  Thr Asn Gly Asn Gly  Lys Leu Ala Leu Trp  Gly Glu His
    1910                 1915                 1920

Thr Gly  Gln Leu Tyr Ser Lys  Phe Leu Leu Lys Ala  Glu Pro Leu
    1925                 1930                 1935

Ala Phe  Thr Phe Ser His Asp  Tyr Lys Gly Ser Thr  Ser His His
    1940                 1945                 1950

Leu Val  Ser Arg Lys Ser Ile  Ser Ala Ala Leu Glu  His Lys Val
    1955                 1960                 1965

Ser Ala  Leu Leu Thr Pro Ala  Glu Gln Thr Gly Thr  Trp Lys Leu
    1970                 1975                 1980

Lys Thr  Gln Phe Asn Asn Asn  Glu Tyr Ser Gln Asp  Leu Asp Ala
    1985                 1990                 1995

Tyr Asn  Thr Lys Asp Lys Ile  Gly Val Glu Leu Thr  Gly Arg Thr
    2000                 2005                 2010

Leu Ala  Asp Leu Thr Leu Leu  Asp Ser Pro Ile Lys  Val Pro Leu
    2015                 2020                 2025

Leu Leu  Ser Glu Pro Ile Asn  Ile Ile Asp Ala Leu  Glu Met Arg
    2030                 2035                 2040

Asp Ala  Val Glu Lys Pro Gln  Glu Phe Thr Ile Val  Ala Phe Val
    2045                 2050                 2055

Lys Tyr  Asp Lys Asn Gln Asp  Val His Ser Ile Asn  Leu Pro Phe
    2060                 2065                 2070

Phe Glu  Thr Leu Gln Glu Tyr  Phe Glu Arg Asn Arg  Gln Thr Ile
    2075                 2080                 2085

Ile Val  Val Leu Glu Asn Val  Gln Arg Asn Leu Lys  His Ile Asn
    2090                 2095                 2100
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Ile Asp  Gln Phe Val Arg Lys  Tyr Arg Ala Ala Leu  Gly Lys Leu
    2105                 2110                 2115

Pro Gln  Gln Ala Asn Asp Tyr  Leu Asn Ser Phe Asn  Trp Glu Arg
    2120                 2125                 2130

Gln Val  Ser His Ala Lys Glu  Lys Leu Thr Ala Leu  Thr Lys Lys
    2135                 2140                 2145

Tyr Arg  Ile Thr Glu Asn Asp  Ile Gln Ile Ala Leu  Asp Asp Ala
    2150                 2155                 2160

Lys Ile  Asn Phe Asn Glu Lys  Leu Ser Gln Leu Gln  Thr Tyr Met
    2165                 2170                 2175

Ile Gln  Phe Asp Gln Tyr Ile  Lys Asp Ser Tyr Asp  Leu His Asp
    2180                 2185                 2190

Leu Lys  Ile Ala Ile Ala Asn  Ile Ile Asp Glu Ile  Ile Glu Lys
    2195                 2200                 2205

Leu Lys  Ser Leu Asp Glu His  Tyr His Ile Arg Val  Asn Leu Val
    2210                 2215                 2220

Lys Thr  Ile His Asp Leu His  Leu Phe Ile Glu Asn  Ile Asp Phe
    2225                 2230                 2235

Asn Lys  Ser Gly Ser Ser Thr  Ala Ser Trp Ile Gln  Asn Val Asp
    2240                 2245                 2250

Thr Lys  Tyr Gln Ile Arg Ile  Gln Ile Gln Glu Lys  Leu Gln Gln
    2255                 2260                 2265

Leu Lys  Arg His Ile Gln Asn  Ile Asp Ile Gln His  Leu Ala Gly
    2270                 2275                 2280

Lys Leu  Lys Gln His Ile Glu  Ala Ile Asp Val Arg  Val Leu Leu
    2285                 2290                 2295

Asp Gln  Leu Gly Thr Thr Ile  Ser Phe Glu Arg Ile  Asn Asp Val
    2300                 2305                 2310

Leu Glu  His Val Lys His Phe  Val Ile Asn Leu Ile  Gly Asp Phe
    2315                 2320                 2325

Glu Val  Ala Glu Lys Ile Asn  Ala Phe Arg Ala Lys  Val His Glu
    2330                 2335                 2340
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Leu Ile  Glu Arg Tyr Glu Val  Asp Gln Gln Ile Gln  Val Leu Met
    2345                 2350                 2355

Asp Lys  Leu Val Glu Leu Ala  His Gln Tyr Lys Leu  Lys Glu Thr
    2360                 2365                 2370

Ile Gln  Lys Leu Ser Asn Val  Leu Gln Gln Val Lys  Ile Lys Asp
    2375                 2380                 2385

Tyr Phe  Glu Lys Leu Val Gly  Phe Ile Asp Asp Ala  Val Lys Lys
    2390                 2395                 2400

Leu Asn  Glu Leu Ser Phe Lys  Thr Phe Ile Glu Asp  Val Asn Lys
    2405                 2410                 2415

Phe Leu  Asp Met Leu Ile Lys  Lys Leu Lys Ser Phe  Asp Tyr His
    2420                 2425                 2430

Gln Phe  Val Asp Glu Thr Asn  Asp Lys Ile Arg Glu  Val Thr Gln
    2435                 2440                 2445

Arg Leu  Asn Gly Glu Ile Gln  Ala Leu Glu Leu Pro  Gln Lys Ala
    2450                 2455                 2460

Glu Ala  Leu Lys Leu Phe Leu  Glu Glu Thr Lys Ala  Thr Val Ala
    2465                 2470                 2475

Val Tyr  Leu Glu Ser Leu Gln  Asp Thr Lys Ile Thr  Leu Ile Ile
    2480                 2485                 2490

Asn Trp  Leu Gln Glu Ala Leu  Ser Ser Ala Ser Leu  Ala His Met
    2495                 2500                 2505

Lys Ala  Lys Phe Arg Glu Thr  Leu Glu Asp Thr Arg  Asp Arg Met
    2510                 2515                 2520

Tyr Gln  Met Asp Ile Gln Gln  Glu Leu Gln Arg Tyr  Leu Ser Leu
    2525                 2530                 2535

Val Gly  Gln Val Tyr Ser Thr  Leu Val Thr Tyr Ile  Ser Asp Trp
    2540                 2545                 2550

Trp Thr  Leu Ala Ala Lys Asn  Leu Thr Asp Phe Ala  Glu Gln Tyr
    2555                 2560                 2565

Ser Ile  Gln Asp Trp Ala Lys  Arg Met Lys Ala Leu  Val Glu Gln
    2570                 2575                 2580
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Gly Phe  Thr Val Pro Glu Ile  Lys Thr Ile Leu Gly  Thr Met Pro
    2585                 2590                 2595

Ala Phe  Glu Val Ser Leu Gln  Ala Leu Gln Lys Ala  Thr Phe Gln
    2600                 2605                 2610

Thr Pro  Asp Phe Ile Val Pro  Leu Thr Asp Leu Arg  Ile Pro Ser
    2615                 2620                 2625

Val Gln  Ile Asn Phe Lys Asp  Leu Lys Asn Ile Lys  Ile Pro Ser
    2630                 2635                 2640

Arg Phe  Ser Thr Pro Glu Phe  Thr Ile Leu Asn Thr  Phe His Ile
    2645                 2650                 2655

Pro Ser  Phe Thr Ile Asp Phe  Val Glu Met Lys Val  Lys Ile Ile
    2660                 2665                 2670

Arg Thr  Ile Asp Gln Met Leu  Asn Ser Glu Leu Gln  Trp Pro Val
    2675                 2680                 2685

Pro Asp  Ile Tyr Leu Arg Asp  Leu Lys Val Glu Asp  Ile Pro Leu
    2690                 2695                 2700

Ala Arg  Ile Thr Leu Pro Asp  Phe Arg Leu Pro Glu  Ile Ala Ile
    2705                 2710                 2715

Pro Glu  Phe Ile Ile Pro Thr  Leu Asn Leu Asn Asp  Phe Gln Val
    2720                 2725                 2730

Pro Asp  Leu His Ile Pro Glu  Phe Gln Leu Pro His  Ile Ser His
    2735                 2740                 2745

Thr Ile  Glu Val Pro Thr Phe  Gly Lys Leu Tyr Ser  Ile Leu Lys
    2750                 2755                 2760

Ile Gln  Ser Pro Leu Phe Thr  Leu Asp Ala Asn Ala  Asp Ile Gly
    2765                 2770                 2775

Asn Gly  Thr Thr Ser Ala Asn  Glu Ala Gly Ile Ala  Ala Ser Ile
    2780                 2785                 2790

Thr Ala  Lys Gly Glu Ser Lys  Leu Glu Val Leu Asn  Phe Asp Phe
    2795                 2800                 2805

Gln Ala  Asn Ala Gln Leu Ser  Asn Pro Lys Ile Asn  Pro Leu Ala
    2810                 2815                 2820
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Leu Lys Glu Ser Val Lys Phe Ser Ser Lys Tyr Leu Arg Thr Glu
2825 2830 2835

His Gly Ser Glu Met Leu Phe Phe Gly Asn Ala Ile Glu Gly Lys
2840 2845 2850

Ser Asn Thr Val Ala Ser Leu His Thr Glu Lys Asn Thr Leu Glu
2855 2860 2865

Leu Ser Asn Gly Val Ile Val Lys Ile Asn Asn Gln Leu Thr Leu
2870 2875 2880

Asp Ser Asn Thr Lys Tyr Phe His Lys Leu Asn Ile Pro Lys Leu
2885 2890 2895

Asp Phe Ser Ser Gln Ala Asp Leu Arg Asn Glu Ile Lys Thr Leu
2900 2905 2910

Leu Lys Ala Gly His Ile Ala Trp Thr Ser Ser Gly Lys Gly Ser
2915 2920 2925

Trp Lys Trp Ala Cys Pro Arg Phe Ser Asp Glu Gly Thr His Glu
2930 2935 2940

Ser Gln Ile Ser Phe Thr Ile Glu Gly Pro Leu Thr Ser Phe Gly
2945 2950 2955

Leu Ser Asn Lys Ile Asn Ser Lys His Leu Arg Val Asn Gln Asn
2960 2965 2970

Leu Val Tyr Glu Ser Gly Ser Leu Asn Phe Ser Lys Leu Glu Ile
2975 2980 2985

Gln Ser Gln Val Asp Ser Gln His Val Gly His Ser Val Leu Thr
2990 2995 3000

Ala Lys Gly Met Ala Leu Phe Gly Glu Gly Lys Ala Glu Phe Thr
3005 3010 3015

Gly Arg His Asp Ala His Leu Asn Gly Lys Val Ile Gly Thr Leu
3020 3025 3030

Lys Asn Ser Leu Phe Phe Ser Ala Gln Pro Phe Glu Ile Thr Ala
3035 3040 3045

Ser Thr Asn Asn Glu Gly Asn Leu Lys Val Arg Phe Pro Leu Arg
3050 3055 3060

Leu Thr Gly Lys Ile Asp Phe Leu Asn Asn Tyr Ala Leu Phe Leu
3065 3070 3075

Ser Pro Ser Ala Gln Gln Ala Ser Trp Gln Val Ser Ala Arg Phe
3080 3085 3090

Asn Gln Tyr Lys Tyr Asn Gln Asn Phe Ser Ala Gly Asn Asn Glu
3095 3100 3105

Asn Ile Met Glu Ala His Val Gly Ile Asn Gly Glu Ala Asn Leu
3110 3115 3120

Asp Phe Leu Asn Ile Pro Leu Thr Ile Pro Glu Met Arg Leu Pro
3125 3130 3135

Tyr Thr Ile Ile Thr Thr Pro Pro Leu Lys Asp Phe Ser Leu Trp
3140 3145 3150

Glu Lys Thr Gly Leu Lys Glu Phe Leu Lys Thr Thr Lys Gln Ser
3155 3160 3165

Phe Asp Leu Ser Val Lys Ala Gln Tyr Lys Lys Asn Lys His Arg
3170 3175 3180

His Ser Ile Thr Asn Pro Leu Ala Val Leu Cys Glu Phe Ile Ser
3185 3190 3195

Gln Ser Ile Lys Ser Phe Asp Arg His Phe Glu Lys Asn Arg Asn
3200 3205 3210

Asn Ala Leu Asp Phe Val Thr Lys Ser Tyr Asn Glu Thr Lys Ile
3215 3220 3225

Lys Phe Asp Lys Tyr Lys Ala Glu Lys Ser His Asp Glu Leu Pro
3230 3235 3240

Arg Thr Phe Gln Ile Pro Gly Tyr Thr Val Pro Val Val Asn Val
3245 3250 3255

Glu Val Ser Pro Phe Thr Ile Glu Met Ser Ala Phe Gly Tyr Val
3260 3265 3270

Phe Pro Lys Ala Val Ser Met Pro Ser Phe Ser Ile Leu Gly Ser
3275 3280 3285

Asp Val Arg Val Pro Ser Tyr Thr Leu Ile Leu Pro Ser Leu Glu
3290 3295 3300

Leu Pro Val Leu His Val Pro Arg Asn Leu Lys Leu Ser Leu Pro
3305 3310 3315

His Phe Lys Glu Leu Cys Thr Ile Ser His Ile Phe Ile Pro Ala
3320 3325 3330

Met Gly Asn Ile Thr Tyr Asp Phe Ser Phe Lys Ser Ser Val Ile
3335 3340 3345

Thr Leu Asn Thr Asn Ala Glu Leu Phe Asn Gln Ser Asp Ile Val
3350 3355 3360

Ala His Leu Leu Ser Ser Ser Ser Ser Val Ile Asp Ala Leu Gln
3365 3370 3375

Tyr Lys Leu Glu Gly Thr Thr Arg Leu Thr Arg Lys Arg Gly Leu
3380 3385 3390

Lys Leu Ala Thr Ala Leu Ser Leu Ser Asn Lys Phe Val Glu Gly
3395 3400 3405

Ser His Asn Ser Thr Val Ser Leu Thr Thr Lys Asn Met Glu Val
3410 3415 3420

Ser Val Ala Lys Thr Thr Lys Ala Glu Ile Pro Ile Leu Arg Met
3425 3430 3435

Asn Phe Lys Gln Glu Leu Asn Gly Asn Thr Lys Ser Lys Pro Thr
3440 3445 3450

Val Ser Ser Ser Met Glu Phe Lys Tyr Asp Phe Asn Ser Ser Met
3455 3460 3465

Leu Tyr Ser Thr Ala Lys Gly Ala Val Asp His Lys Leu Ser Leu
3470 3475 3480

Glu Ser Leu Thr Ser Tyr Phe Ser Ile Glu Ser Ser Thr Lys Gly
3485 3490 3495

Asp Val Lys Gly Ser Val Leu Ser Arg Glu Tyr Ser Gly Thr Ile
3500 3505 3510

Ala Ser Glu Ala Asn Thr Tyr Leu Asn Ser Lys Ser Thr Arg Ser
3515 3520 3525

Ser Val Lys Leu Gln Gly Thr Ser Lys Ile Asp Asp Ile Trp Asn
3530 3535 3540

Leu Glu Val Lys Glu Asn Phe Ala Gly Glu Ala Thr Leu Gln Arg
3545 3550 3555

Ile Tyr Ser Leu Trp Glu His Ser Thr Lys Asn His Leu Gln Leu
3560 3565 3570

Glu Gly Leu Phe Phe Thr Asn Gly Glu His Thr Ser Lys Ala Thr
3575 3580 3585

Leu Glu Leu Ser Pro Trp Gln Met Ser Ala Leu Val Gln Val His
3590 3595 3600

Ala Ser Gln Pro Ser Ser Phe His Asp Phe Pro Asp Leu Gly Gln
3605 3610 3615

Glu Val Ala Leu Asn Ala Asn Thr Lys Asn Gln Lys Ile Arg Trp
3620 3625 3630

Lys Asn Glu Val Arg Ile His Ser Gly Ser Phe Gln Ser Gln Val
3635 3640 3645

Glu Leu Ser Asn Asp Gln Glu Lys Ala His Leu Asp Ile Ala Gly
3650 3655 3660

Ser Leu Glu Gly His Leu Arg Phe Leu Lys Asn Ile Ile Leu Pro
3665 3670 3675

Val Tyr Asp Lys Ser Leu Trp Asp Phe Leu Lys Leu Asp Val Thr
3680 3685 3690

Thr Ser Ile Gly Arg Arg Gln His Leu Arg Val Ser Thr Ala Phe
3695 3700 3705

Val Tyr Thr Lys Asn Pro Asn Gly Tyr Ser Phe Ser Ile Pro Val
3710 3715 3720

Lys Val Leu Ala Asp Lys Phe Ile Thr Pro Gly Leu Lys Leu Asn
3725 3730 3735

Asp Leu Asn Ser Val Leu Val Met Pro Thr Phe His Val Pro Phe
3740 3745 3750

Thr Asp Leu Gln Val Pro Ser Cys Lys Leu Asp Phe Arg Glu Ile
3755 3760 3765

Gln Ile Tyr Lys Lys Leu Arg Thr Ser Ser Phe Ala Leu Asn Leu
3770 3775 3780

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Pro Thr  Leu Pro Glu Val Lys  Phe Pro Glu Val Asp  Val Leu Thr
    3785                 3790                 3795

Lys Tyr  Ser Gln Pro Glu Asp  Ser Leu Ile Pro Phe  Phe Glu Ile
    3800                 3805                 3810

Thr Val  Pro Glu Ser Gln Leu  Thr Val Ser Gln Phe  Thr Leu Pro
    3815                 3820                 3825

Lys Ser  Val Ser Asp Gly Ile  Ala Ala Leu Asp Leu  Asn Ala Val
    3830                 3835                 3840

Ala Asn  Lys Ile Ala Asp Phe  Glu Leu Pro Thr Ile  Ile Val Pro
    3845                 3850                 3855

Glu Gln  Thr Ile Glu Ile Pro  Ser Ile Lys Phe Ser  Val Pro Ala
    3860                 3865                 3870

Gly Ile  Val Ile Pro Ser Phe  Gln Ala Leu Thr Ala  Arg Phe Glu
    3875                 3880                 3885

Val Asp  Ser Pro Val Tyr Asn  Ala Thr Trp Ser Ala  Ser Leu Lys
    3890                 3895                 3900

Asn Lys  Ala Asp Tyr Val Glu  Thr Val Leu Asp Ser  Thr Cys Ser
    3905                 3910                 3915

Ser Thr  Val Gln Phe Leu Glu  Tyr Glu Leu Asn Val  Leu Gly Thr
    3920                 3925                 3930

His Lys  Ile Glu Asp Gly Thr  Leu Ala Ser Lys Thr  Lys Gly Thr
    3935                 3940                 3945

Leu Ala  His Arg Asp Phe Ser  Ala Glu Tyr Glu Glu  Asp Gly Lys
    3950                 3955                 3960

Phe Glu  Gly Leu Gln Glu Trp  Glu Gly Lys Ala His  Leu Asn Ile
    3965                 3970                 3975

Lys Ser  Pro Ala Phe Thr Asp  Leu His Leu Arg Tyr  Gln Lys Asp
    3980                 3985                 3990

Lys Lys  Gly Ile Ser Thr Ser  Ala Ala Ser Pro Ala  Val Gly Thr
    3995                 4000                 4005

Val Gly  Met Asp Met Asp Glu  Asp Asp Asp Phe Ser  Lys Trp Asn
    4010                 4015                 4020
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Phe Tyr  Tyr Ser Pro Gln Ser  Ser Pro Asp Lys Lys  Leu Thr Ile
    4025                 4030                 4035

Phe Lys  Thr Glu Leu Arg Val  Arg Glu Ser Asp Glu  Glu Thr Gln
    4040                 4045                 4050

Ile Lys  Val Asn Trp Glu Glu  Glu Ala Ala Ser Gly  Leu Leu Thr
    4055                 4060                 4065

Ser Leu  Lys Asp Asn Val Pro  Lys Ala Thr Gly Val  Leu Tyr Asp
    4070                 4075                 4080

Tyr Val  Asn Lys Tyr His Trp  Glu His Thr Gly Leu  Thr Leu Arg
    4085                 4090                 4095

Glu Val  Ser Ser Lys Leu Arg  Arg Asn Leu Gln Asn  Asn Ala Glu
    4100                 4105                 4110

Trp Val  Tyr Gln Gly Ala Ile  Arg Gln Ile Asp Asp  Ile Asp Val
    4115                 4120                 4125

Arg Phe  Gln Lys Ala Ala Ser  Gly Thr Thr Gly Thr  Tyr Gln Glu
    4130                 4135                 4140

Trp Lys  Asp Lys Ala Gln Asn  Leu Tyr Gln Glu Leu  Leu Thr Gln
    4145                 4150                 4155

Glu Gly  Gln Ala Ser Phe Gln  Gly Leu Lys Asp Asn  Val Phe Asp
    4160                 4165                 4170

Gly Leu  Val Arg Val Thr Gln  Lys Phe His Met Lys  Val Lys His
    4175                 4180                 4185

Leu Ile  Asp Ser Leu Ile Asp  Phe Leu Asn Phe Pro  Arg Phe Gln
    4190                 4195                 4200

Phe Pro  Gly Lys Pro Gly Ile  Tyr Thr Arg Glu Glu  Leu Cys Thr
    4205                 4210                 4215

Met Phe  Ile Arg Glu Val Gly  Thr Val Leu Ser Gln  Val Tyr Ser
    4220                 4225                 4230

Lys Val  His Asn Gly Ser Glu  Ile Leu Phe Ser Tyr  Phe Gln Asp
    4235                 4240                 4245

Leu Val  Ile Thr Leu Pro Phe  Glu Leu Arg Lys His  Lys Leu Ile
    4250                 4255                 4260
```

```
Asp Val  Ile Ser Met Tyr Arg  Glu Leu Leu Lys Asp  Leu Ser Lys
    4265                 4270                 4275

Glu Ala  Gln Glu Val Phe Lys  Ala Ile Gln Ser Leu  Lys Thr Thr
    4280                 4285                 4290

Glu Val  Leu Arg Asn Leu Gln  Asp Leu Leu Gln Phe  Ile Phe Gln
    4295                 4300                 4305

Leu Ile  Glu Asp Asn Ile Lys  Gln Leu Lys Glu Met  Lys Phe Thr
    4310                 4315                 4320

Tyr Leu  Ile Asn Tyr Ile Gln  Asp Glu Ile Asn Thr  Ile Phe Asn
    4325                 4330                 4335

Asp Tyr  Ile Pro Tyr Val Phe  Lys Leu Leu Lys Glu  Asn Leu Cys
    4340                 4345                 4350

Leu Asn  Leu His Lys Phe Asn  Glu Phe Ile Gln Asn  Glu Leu Gln
    4355                 4360                 4365

Glu Ala  Ser Gln Glu Leu Gln  Gln Ile His Gln Tyr  Ile Met Ala
    4370                 4375                 4380

Leu Arg  Glu Glu Tyr Phe Asp  Pro Ser Ile Val Gly  Trp Thr Val
    4385                 4390                 4395

Lys Tyr  Tyr Glu Leu Glu Glu  Lys Ile Val Ser Leu  Ile Lys Asn
    4400                 4405                 4410

Leu Leu  Val Ala Leu Lys Asp  Phe His Ser Glu Tyr  Ile Val Ser
    4415                 4420                 4425

Ala Ser  Asn Phe Thr Ser Gln  Leu Ser Ser Gln Val  Glu Gln Phe
    4430                 4435                 4440

Leu His  Arg Asn Ile Gln Glu  Tyr Leu Ser Ile Leu  Thr Asp Pro
    4445                 4450                 4455

Asp Gly  Lys Gly Lys Glu Lys  Ile Ala Glu Leu Ser  Ala Thr Ala
    4460                 4465                 4470

Gln Glu  Ile Ile Lys Ser Gln  Ala Ile Ala Thr Lys  Lys Ile Ile
    4475                 4480                 4485

Ser Asp  Tyr His Gln Gln Phe  Arg Tyr Lys Leu Gln  Asp Phe Ser
    4490                 4495                 4500
```

```
Asp Gln  Leu Ser Asp Tyr Tyr  Glu Lys Phe Ile Ala  Glu Ser Lys
    4505                 4510                 4515

Arg Leu  Ile Asp Leu Ser Ile  Gln Asn Tyr His Thr  Phe Leu Ile
    4520                 4525                 4530

Tyr Ile  Thr Glu Leu Leu Lys  Lys Leu Gln Ser Thr  Thr Val Met
    4535                 4540                 4545

Asn Pro  Tyr Met Lys Leu Ala  Pro Gly Glu Leu Thr  Ile Ile Leu
    4550                 4555                 4560
```

<210> 26
<211> 317
<212> PRT
<213> Homo sapiens

<220> misc_feature
<222> (162)..(165)
<223> heparin binding domain

<220> misc_feature
<222> (229)..(236)
<223> heparin binding domain

<400> 26

```
Met Lys Val Leu Trp Ala Ala Leu Leu Val Thr Phe Leu Ala Gly Cys
1               5                  10                  15

Gln Ala Lys Val Glu Gln Ala Val Glu Thr Glu Pro Glu Pro Glu Leu
            20                  25                  30

Arg Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu
        35                  40                  45

Gly Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln
    50                  55                  60

Val Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala
65                  70                  75                  80

Leu Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu
                85                  90                  95

Glu Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser
            100                 105                 110

Lys Glu Leu Gln Ala Ala Gln Ala Arg Leu Gly Ala Asp Met Glu Asp
        115                 120                 125
```

```
Val Cys Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu
    130                 135                 140

Gly Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg
145                 150                 155                 160

Lys Leu Arg Lys Arg Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg
                165                 170                 175

Leu Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu
            180                 185                 190

Ser Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val
        195                 200                 205

Arg Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg
    210                 215                 220

Ala Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly
225                 230                 235                 240

Ser Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu
                245                 250                 255

Val Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala
            260                 265                 270

Glu Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu
        275                 280                 285

Asp Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala
    290                 295                 300

Val Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
305                 310                 315
```

<210> 27
<211> 268
<212> PRT
<213> Homo sapiens

<400> 27

```
Met Ser Leu Ser Phe Leu Leu Leu Leu Phe Phe Ser His Leu Ile Leu
1               5                   10                  15

Ser Ala Trp Ala His Gly Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro
            20                  25                  30
```

```
Gly Pro Ala Ala Thr Asp Arg Asn Pro Arg Gly Ser Ser Ser Arg Gln
        35                  40                  45

Ser Ser Ser Ser Ala Met Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala
    50                  55                  60

Ala Ser Leu Gly Ser Gln Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln
65                  70                  75                  80

Trp Ser Pro Ser Gly Arg Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly
                85                  90                  95

Ile Gly Phe His Leu Gln Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser
            100                 105                 110

His Glu Ala Asn Met Leu Ser Val Leu Glu Ile Phe Ala Val Ser Gln
        115                 120                 125

Gly Ile Val Gly Ile Arg Gly Val Phe Ser Asn Lys Phe Leu Ala Met
    130                 135                 140

Ser Lys Lys Gly Lys Leu His Ala Ser Ala Lys Phe Thr Asp Asp Cys
145                 150                 155                 160

Lys Phe Arg Glu Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser
                165                 170                 175

Ala Ile His Arg Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu
            180                 185                 190

Asn Lys Arg Gly Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro
        195                 200                 205

Gln His Ile Ser Thr His Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln
    210                 215                 220

Pro Glu Leu Ser Phe Thr Val Thr Val Pro Glu Lys Lys Asn Pro Pro
225                 230                 235                 240

Ser Pro Ile Lys Ser Lys Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr
                245                 250                 255

Asn Ser Val Lys Tyr Arg Leu Lys Phe Arg Phe Gly
            260                 265
```

<210> 28
<211> 55
<212> PRT

<213> Homo sapiens

<400> 28

```
Ala Arg Gln Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His
1               5                  10                  15

Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr
            20                  25                  30

Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys
        35                  40                  45

Arg Cys Asp Lys Pro Arg Arg
    50                  55
```

<210> 29
<211> 271
<212> PRT
<213> Homo sapiens

<400> 29

```
Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro Thr
1               5                  10                  15

Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr
            20                  25                  30

Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met Lys Glu Ile
        35                  40                  45

Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val
    50                  55                  60

Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr
65                  70                  75                  80

Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro
                85                  90                  95

Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile
            100                 105                 110

Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala
        115                 120                 125

Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp
    130                 135                 140
```

```
Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys
145                 150                 155                 160

Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val
                165                 170                 175

Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu
            180                 185                 190

Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala
        195                 200                 205

Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro
    210                 215                 220

Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile
225                 230                 235                 240

Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala Leu
                245                 250                 255

Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys Thr
            260                 265                 270
```

<210> 30
<211> 225
<212> PRT
<213> Homo sapiens

<400> 30

```
Ile Val Gly Gly Arg Lys Ala Arg Pro Arg Gln Phe Pro Phe Leu Ala
1               5                   10                  15

Ser Ile Gln Asn Gln Gly Arg His Phe Cys Gly Gly Ala Leu Ile His
            20                  25                  30

Ala Arg Phe Val Met Thr Ala Ala Ser Cys Phe Gln Ser Gln Asn Pro
        35                  40                  45

Gly Val Ser Thr Val Val Leu Gly Ala Tyr Asp Leu Arg Arg Arg Glu
    50                  55                  60

Arg Gln Ser Arg Gln Thr Phe Ser Ile Ser Ser Met Ser Glu Asn Gly
65                  70                  75                  80

Tyr Asp Pro Gln Gln Asn Leu Asn Asp Leu Met Leu Leu Gln Leu Asp
                85                  90                  95
```

```
Arg Glu Ala Asn Leu Thr Ser Ser Val Thr Ile Leu Pro Leu Pro Leu
            100                 105                 110

Gln Asn Ala Thr Val Glu Ala Gly Thr Arg Cys Gln Val Ala Gly Trp
        115                 120                 125

Gly Ser Gln Arg Ser Gly Gly Arg Leu Ser Arg Phe Pro Arg Phe Val
    130                 135                 140

Asn Val Thr Val Thr Pro Glu Asp Gln Cys Arg Pro Asn Asn Val Cys
145                 150                 155                 160

Thr Gly Val Leu Thr Arg Arg Gly Gly Ile Cys Asn Gly Asp Gly Gly
                165                 170                 175

Thr Pro Leu Val Cys Glu Gly Leu Ala His Gly Val Ala Ser Phe Ser
            180                 185                 190

Leu Gly Pro Cys Gly Arg Gly Pro Asp Phe Phe Thr Arg Val Ala Leu
        195                 200                 205

Phe Arg Asp Trp Ile Asp Gly Val Leu Asn Asn Pro Gly Pro Gly Pro
    210                 215                 220

Ala
225
```

<210> 31
<211> 374
<212> PRT
<213> Homo sapiens

<400> 31

```
Ala Val Thr Thr Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val
1               5                   10                  15

Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu
            20                  25                  30

Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
        35                  40                  45

Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly
    50                  55                  60

Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp
65                  70                  75                  80
```

```
Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn
                85                  90                  95

Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr
            100                 105                 110

Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln
        115                 120                 125

Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile
    130                 135                 140

Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr
145                 150                 155                 160

Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser
                165                 170                 175

Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu
            180                 185                 190

Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln Pro
        195                 200                 205

Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly
    210                 215                 220

Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu
225                 230                 235                 240

Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val
                245                 250                 255

Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys
            260                 265                 270

Lys Thr Gly Gln Glu Ala Leu Ser Gln Thr Thr Ile Ser Trp Ala Pro
        275                 280                 285

Phe Gln Asp Thr Ser Glu Tyr Ile Ile Ser Cys His Pro Val Gly Thr
    290                 295                 300

Asp Glu Glu Pro Leu Gln Phe Val Pro Gly Thr Ser Thr Ser Ala Thr
305                 310                 315                 320

Leu Thr Gly Leu Thr Arg Gly Ala Thr Tyr Asn Ile Ile Val Glu Ala
                325                 330                 335
```

Leu Lys Asp Gln Gln Arg His Lys Val Arg Glu Glu Val Val Thr Val
340 345 350

Gly Asn Ser Val Asn Glu Gly Leu Asn Gln Pro Thr Asp Asp Ser Cys
355 360 365

Phe Asp Pro Tyr Thr Val
370

<210> 32
<211> 51
<212> PRT
<213> Homo sapiens

<400> 32

Cys His Pro Gly Tyr His Gly Glu Arg Cys His Gly Leu Ser Leu Pro
1 5 10 15

Val Glu Asn His Leu Tyr Thr Tyr Asp His Thr Thr Ile Leu Ala Val
20 25 30

Val Ala Val Val Leu Ser Ser Val Cys Leu Leu Val Ile Val Gly Leu
35 40 45

Leu Met Phe
50

<210> 33
<211> 23
<212> PRT
<213> Homo sapiens

<400> 33

Tyr His Arg Arg Gly Gly Tyr Asp Val Glu Asn Glu Glu Lys Val Lys
1 5 10 15

Leu Gly Met Thr Asn Ser His
20

<210> 34
<211> 58
<212> PRT
<213> Homo sapiens

<400> 34

Phe Ile Leu Gln Ala Thr Ser Thr Gly His Thr Lys Gln Gly Gly Ala
1 5 10 15

Arg Glu Lys Lys Glu Glu Arg Gln Gly Ala Arg Glu Glu Glu Gly Pro
20 25 30

```
Met Ser Ser Glu Ile Gln Gly Leu Leu His Pro Trp Arg Met Gln Ile
        35                  40                  45

Cys Glu Gly Ala Pro Gly Ser Leu Leu His
    50                  55

<210>  35
<211>  317
<212>  PRT
<213>  Homo sapiens

<220>  misc_feature
<222>  (162)..(165)
<223>  heparin binding domain

<220>  misc_feature
<222>  (229)..(236)
<223>  heparin binding domain

<400>  35

Met Lys Val Leu Trp Ala Ala Leu Leu Val Thr Phe Leu Ala Gly Cys
1               5                   10                  15

Gln Ala Lys Val Glu Gln Ala Val Glu Thr Glu Pro Glu Pro Glu Leu
            20                  25                  30

Arg Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu
        35                  40                  45

Gly Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln
    50                  55                  60

Val Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala
65                  70                  75                  80

Leu Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu
                85                  90                  95

Glu Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser
            100                 105                 110

Lys Glu Leu Gln Ala Ala Gln Ala Arg Leu Gly Ala Asp Met Glu Asp
        115                 120                 125

Val Cys Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu
    130                 135                 140

Gly Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg
145                 150                 155                 160
```

```
Lys Leu Arg Lys Arg Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg
                165                 170                 175

Leu Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu
            180                 185                 190

Ser Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val
        195                 200                 205

Arg Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg
    210                 215                 220

Ala Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly
225                 230                 235                 240

Ser Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu
                245                 250                 255

Val Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala
            260                 265                 270

Glu Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu
        275                 280                 285

Asp Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala
    290                 295                 300

Val Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
305                 310                 315
```

<210> 36
<211> 316
<212> PRT
<213> Homo sapiens

<400> 36

```
Met Arg Gln Pro Leu Ser Trp Gly Arg Trp Arg Ala Met Leu Ala Arg
1               5                   10                  15

Thr Tyr Gly Pro Gly Pro Ser Ala Gly Tyr Arg Trp Ala Ser Gly Ala
            20                  25                  30

Gln Gly Tyr Val Arg Asn Pro Pro Val Gly Ala Cys Asp Leu Gln Gly
        35                  40                  45

Glu Leu Asp Arg Phe Gly Gly Ile Ser Val Arg Leu Ala Arg Leu Asp
    50                  55                  60
```

```
Ala Leu Asp Arg Leu Asp Ala Ala Ala Phe Gln Lys Gly Leu Gln Ala
65                  70                  75                  80

Ala Val Gln Gln Trp Arg Ser Glu Gly Arg Thr Ala Val Trp Leu His
                85                  90                  95

Ile Pro Ile Leu Gln Ser Arg Phe Ile Ala Pro Ala Ala Ser Leu Gly
            100                 105                 110

Phe Cys Phe His His Ala Glu Ser Asp Ser Ser Thr Leu Thr Leu Trp
        115                 120                 125

Leu Arg Glu Gly Pro Ser Arg Leu Pro Gly Tyr Ala Ser His Gln Val
    130                 135                 140

Gly Val Ala Gly Ala Val Phe Asp Glu Ser Thr Arg Lys Ile Leu Val
145                 150                 155                 160

Val Gln Asp Arg Asn Lys Leu Lys Asn Met Trp Lys Phe Pro Gly Gly
                165                 170                 175

Leu Ser Glu Pro Glu Glu Asp Ile Gly Asp Thr Ala Val Arg Glu Val
            180                 185                 190

Phe Glu Glu Thr Gly Ile Lys Ser Glu Phe Arg Ser Val Leu Ser Ile
        195                 200                 205

Arg Gln Gln His Thr Asn Pro Gly Ala Phe Gly Lys Ser Asp Met Tyr
    210                 215                 220

Ile Ile Cys Arg Leu Lys Pro Tyr Ser Phe Thr Ile Asn Phe Cys Gln
225                 230                 235                 240

Glu Glu Cys Leu Arg Cys Glu Trp Met Asp Leu Asn Asp Leu Ala Lys
                245                 250                 255

Thr Glu Asn Thr Thr Pro Ile Thr Ser Arg Val Ala Arg Leu Leu Leu
            260                 265                 270

Tyr Gly Tyr Arg Glu Gly Phe Asp Lys Ile Asp Leu Thr Val Glu Glu
        275                 280                 285

Leu Pro Ala Val Tyr Thr Gly Leu Phe Tyr Lys Leu Tyr His Lys Glu
    290                 295                 300

Leu Pro Glu Asn Tyr Lys Thr Met Lys Gly Ile Asp
305                 310                 315
```

<210> 37
<211> 147
<212> PRT
<213> Homo sapiens

<400> 37

Met Trp Lys Phe Pro Gly Gly Leu Ser Glu Pro Glu Glu Asp Ile Gly
1 5 10 15

Asp Thr Ala Val Arg Glu Val Phe Glu Glu Thr Gly Ile Lys Ser Glu
20 25 30

Phe Arg Ser Val Leu Ser Ile Arg Gln Gln His Thr Asn Pro Gly Ala
35 40 45

Phe Gly Lys Ser Asp Met Tyr Ile Ile Cys Arg Leu Lys Pro Tyr Ser
50 55 60

Phe Thr Ile Asn Phe Cys Gln Glu Glu Cys Leu Arg Cys Glu Trp Met
65 70 75 80

Asp Leu Asn Asp Leu Ala Lys Thr Glu Asn Thr Thr Pro Ile Thr Ser
85 90 95

Arg Val Ala Arg Leu Leu Leu Tyr Gly Tyr Arg Glu Gly Phe Asp Lys
100 105 110

Ile Asp Leu Thr Val Glu Glu Leu Pro Ala Val Tyr Thr Gly Leu Phe
115 120 125

Tyr Lys Leu Tyr His Lys Glu Leu Pro Glu Asn Tyr Lys Thr Met Lys
130 135 140

Gly Ile Asp
145

<210> 38
<211> 251
<212> PRT
<213> Homo sapiens

<400> 38

Met Thr Arg Leu Thr Val Leu Ala Leu Leu Ala Gly Leu Leu Ala Ser
1 5 10 15

Ser Arg Ala Gly Ser Ser Pro Leu Leu Asp Ile Val Gly Gly Arg Lys
20 25 30

Ala Arg Pro Arg Gln Phe Pro Phe Leu Ala Ser Ile Gln Asn Gln Gly

35 40 45

Arg His Phe Cys Gly Gly Ala Leu Ile His Ala Arg Phe Val Met Thr
50 55 60

Ala Ala Ser Cys Phe Gln Ser Gln Asn Pro Gly Val Ser Thr Val Val
65 70 75 80

Leu Gly Ala Tyr Asp Leu Arg Arg Arg Glu Arg Gln Ser Arg Gln Thr
85 90 95

Phe Ser Ile Ser Ser Met Ser Glu Asn Gly Tyr Asp Pro Gln Gln Asn
100 105 110

Leu Asn Asp Leu Met Leu Leu Gln Leu Asp Arg Glu Ala Asn Leu Thr
115 120 125

Ser Ser Val Thr Ile Leu Pro Leu Pro Leu Gln Asn Ala Thr Val Glu
130 135 140

Ala Gly Thr Arg Cys Gln Val Ala Gly Trp Gly Ser Gln Arg Ser Gly
145 150 155 160

Gly Arg Leu Ser Arg Phe Pro Arg Phe Val Asn Val Thr Val Thr Pro
165 170 175

Glu Asp Gln Cys Arg Pro Asn Asn Val Cys Thr Gly Val Leu Thr Arg
180 185 190

Arg Gly Gly Ile Cys Asn Gly Asp Gly Gly Thr Pro Leu Val Cys Glu
195 200 205

Gly Leu Ala His Gly Val Ala Ser Phe Ser Leu Gly Pro Cys Gly Arg
210 215 220

Gly Pro Asp Phe Phe Thr Arg Val Ala Leu Phe Arg Asp Trp Ile Asp
225 230 235 240

Gly Val Leu Asn Asn Pro Gly Pro Gly Pro Ala
245 250

<210> 39
<211> 345
<212> PRT
<213> Homo sapiens

<400> 39

Met Ile Ser Pro Val Leu Ile Leu Phe Ser Ser Phe Leu Cys His Val

```
1               5                   10                  15

Ala Ile Ala Gly Arg Thr Cys Pro Lys Pro Asp Asp Leu Pro Phe Ser
            20                  25                  30

Thr Val Val Pro Leu Lys Thr Phe Tyr Glu Pro Gly Glu Glu Ile Thr
        35                  40                  45

Tyr Ser Cys Lys Pro Gly Tyr Val Ser Arg Gly Gly Met Arg Lys Phe
    50                  55                  60

Ile Cys Pro Leu Thr Gly Leu Trp Pro Ile Asn Thr Leu Lys Cys Thr
65                  70                  75                  80

Pro Arg Val Cys Pro Phe Ala Gly Ile Leu Glu Asn Gly Ala Val Arg
                85                  90                  95

Tyr Thr Thr Phe Glu Tyr Pro Asn Thr Ile Ser Phe Ser Cys Asn Thr
            100                 105                 110

Gly Phe Tyr Leu Asn Gly Ala Asp Ser Ala Lys Cys Thr Glu Glu Gly
        115                 120                 125

Lys Trp Ser Pro Glu Leu Pro Val Cys Ala Pro Ile Ile Cys Pro Pro
    130                 135                 140

Pro Ser Ile Pro Thr Phe Ala Thr Leu Arg Val Tyr Lys Pro Ser Ala
145                 150                 155                 160

Gly Asn Asn Ser Leu Tyr Arg Asp Thr Ala Val Phe Glu Cys Leu Pro
                165                 170                 175

Gln His Ala Met Phe Gly Asn Asp Thr Ile Thr Cys Thr Thr His Gly
            180                 185                 190

Asn Trp Thr Lys Leu Pro Glu Cys Arg Glu Val Lys Cys Pro Phe Pro
        195                 200                 205

Ser Arg Pro Asp Asn Gly Phe Val Asn Tyr Pro Ala Lys Pro Thr Leu
    210                 215                 220

Tyr Tyr Lys Asp Lys Ala Thr Phe Gly Cys His Asp Gly Tyr Ser Leu
225                 230                 235                 240

Asp Gly Pro Glu Glu Ile Glu Cys Thr Lys Leu Gly Asn Trp Ser Ala
                245                 250                 255
```

```
Met Pro Ser Cys Lys Ala Ser Cys Lys Val Pro Val Lys Lys Ala Thr
            260                 265                 270

Val Val Tyr Gln Gly Glu Arg Val Lys Ile Gln Glu Lys Phe Lys Asn
        275                 280                 285

Gly Met Leu His Gly Asp Lys Val Ser Phe Phe Cys Lys Asn Lys Glu
    290                 295                 300

Lys Lys Cys Ser Tyr Thr Glu Asp Ala Gln Cys Ile Asp Gly Thr Ile
305                 310                 315                 320

Glu Val Pro Lys Cys Phe Lys Glu His Ser Ser Leu Ala Phe Trp Lys
                325                 330                 335

Thr Asp Ala Ser Asp Val Lys Pro Cys
            340                 345
```

<210> 40
<211> 464
<212> PRT
<213> Homo sapiens

<400> 40

```
Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val
1               5                   10                  15

Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys
            20                  25                  30

His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro
        35                  40                  45

Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu
    50                  55                  60

Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val
65                  70                  75                  80

Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln
                85                  90                  95

His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro
            100                 105                 110

Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn
        115                 120                 125
```

```
Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser
    130                 135                 140

Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys
145                 150                 155                 160

Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn
                165                 170                 175

Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp
            180                 185                 190

Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys
        195                 200                 205

Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn
    210                 215                 220

Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn
225                 230                 235                 240

Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu
                245                 250                 255

Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr
            260                 265                 270

Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly
        275                 280                 285

Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu
    290                 295                 300

Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro
305                 310                 315                 320

Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu
                325                 330                 335

Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met
            340                 345                 350

Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln
        355                 360                 365

Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro
    370                 375                 380
```

```
Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe
385                 390                 395                 400

His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala
                405                 410                 415

Ser Thr Ala Val Val Ile Ala Gly Arg Ser Leu Asn Pro Asn Arg Val
            420                 425                 430

Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro
        435                 440                 445

Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys
    450                 455                 460
```

<210> 41
<211> 240
<212> PRT
<213> Homo sapiens

<400> 41

```
Met Ser Arg Ser Asn Arg Gln Lys Glu Tyr Lys Cys Gly Asp Leu Val
1               5                   10                  15

Phe Ala Lys Met Lys Gly Tyr Pro His Trp Pro Ala Arg Ile Asp Glu
            20                  25                  30

Met Pro Glu Ala Ala Val Lys Ser Thr Ala Asn Lys Tyr Gln Val Phe
        35                  40                  45

Phe Phe Gly Thr His Glu Thr Ala Phe Leu Gly Pro Lys Asp Leu Phe
    50                  55                  60

Pro Tyr Glu Glu Ser Lys Glu Lys Phe Gly Lys Pro Asn Lys Arg Lys
65                  70                  75                  80

Gly Phe Ser Glu Gly Leu Trp Glu Ile Glu Asn Asn Pro Thr Val Lys
                85                  90                  95

Ala Ser Gly Tyr Gln Ser Ser Gln Lys Lys Ser Cys Val Glu Glu Pro
            100                 105                 110

Glu Pro Glu Pro Glu Ala Ala Glu Gly Asp Gly Asp Lys Lys Gly Asn
        115                 120                 125

Ala Glu Gly Ser Ser Asp Glu Glu Gly Lys Leu Val Ile Asp Glu Pro
    130                 135                 140
```

```
Ala Lys Glu Lys Asn Glu Lys Gly Ala Leu Lys Arg Arg Ala Gly Asp
145                 150                 155                 160

Leu Leu Glu Asp Ser Pro Lys Arg Pro Lys Glu Ala Glu Asn Pro Glu
                165                 170                 175

Gly Glu Glu Lys Glu Ala Ala Thr Leu Glu Val Glu Arg Pro Leu Pro
            180                 185                 190

Met Glu Val Glu Lys Asn Ser Thr Pro Ser Glu Pro Gly Ser Gly Arg
        195                 200                 205

Gly Pro Pro Gln Glu Glu Glu Glu Glu Glu Asp Glu Glu Glu Glu Ala
    210                 215                 220

Thr Lys Glu Asp Ala Glu Ala Pro Gly Ile Arg Asp His Glu Ser Leu
225                 230                 235                 240
```

<210> 42
<211> 525
<212> PRT
<213> Homo sapiens

<400> 42

```
Met Lys Ala Leu Ile Ala Ala Leu Leu Leu Ile Thr Leu Gln Tyr Ser
1               5                   10                  15

Cys Ala Val Ser Pro Thr Asp Cys Ser Ala Val Glu Pro Glu Ala Glu
            20                  25                  30

Lys Ala Leu Asp Leu Ile Asn Lys Arg Arg Arg Asp Gly Tyr Leu Phe
        35                  40                  45

Gln Leu Leu Arg Ile Ala Asp Ala His Leu Asp Arg Val Glu Asn Thr
    50                  55                  60

Thr Val Tyr Tyr Leu Val Leu Asp Val Gln Glu Ser Asp Cys Ser Val
65                  70                  75                  80

Leu Ser Arg Lys Tyr Trp Asn Asp Cys Glu Pro Pro Asp Ser Arg Arg
                85                  90                  95

Pro Ser Glu Ile Val Ile Gly Gln Cys Lys Val Ile Ala Thr Arg His
            100                 105                 110

Ser His Glu Ser Gln Asp Leu Arg Val Ile Asp Phe Asn Cys Thr Thr
        115                 120                 125
```

```
Ser Ser Val Ser Ser Ala Leu Ala Asn Thr Lys Asp Ser Pro Val Leu
    130                 135                 140

Ile Asp Phe Phe Glu Asp Thr Glu Arg Tyr Arg Lys Gln Ala Asn Lys
145                 150                 155                 160

Ala Leu Glu Lys Tyr Lys Glu Glu Asn Asp Asp Phe Ala Ser Phe Arg
                165                 170                 175

Val Asp Arg Ile Glu Arg Val Ala Arg Val Arg Gly Gly Glu Gly Thr
            180                 185                 190

Gly Tyr Phe Val Asp Phe Ser Val Arg Asn Cys Pro Arg His His Phe
        195                 200                 205

Pro Arg His Pro Asn Val Phe Gly Phe Cys Arg Ala Asp Leu Phe Tyr
    210                 215                 220

Asp Val Glu Ala Leu Asp Leu Glu Ser Pro Lys Asn Leu Val Ile Asn
225                 230                 235                 240

Cys Glu Val Phe Asp Pro Gln Glu His Glu Asn Ile Asn Gly Val Pro
                245                 250                 255

Pro His Leu Gly His Pro Phe His Trp Gly Gly His Glu Arg Ser Ser
            260                 265                 270

Thr Thr Lys Pro Pro Phe Lys Pro His Gly Ser Arg Asp His His His
        275                 280                 285

Pro His Lys Pro His Glu His Gly Pro Pro Pro Pro Pro Asp Glu Arg
    290                 295                 300

Asp His Ser His Gly Pro Pro Leu Pro Gln Gly Pro Pro Pro Leu Leu
305                 310                 315                 320

Pro Met Ser Cys Ser Ser Cys Gln His Ala Thr Phe Gly Thr Asn Gly
                325                 330                 335

Ala Gln Arg His Ser His Asn Asn Asn Ser Ser Asp Leu His Pro His
            340                 345                 350

Lys His His Ser His Glu Gln His Pro His Gly His His Pro His Ala
        355                 360                 365

His His Pro His Glu His Asp Thr His Arg Gln His Pro His Gly His
    370                 375                 380
```

```
His Pro His Gly His His Pro His Gly His His Pro His Gly His His
385                 390                 395                 400

Pro His Gly His His Pro His Cys His Asp Phe Gln Asp Tyr Gly Pro
                405                 410                 415

Cys Asp Pro Pro Pro His Asn Gln Gly His Cys Cys His Gly His Gly
            420                 425                 430

Pro Pro Pro Gly His Leu Arg Arg Arg Gly Pro Gly Lys Gly Pro Arg
        435                 440                 445

Pro Phe His Cys Arg Gln Ile Gly Ser Val Tyr Arg Leu Pro Pro Leu
    450                 455                 460

Arg Lys Gly Glu Val Leu Pro Leu Pro Glu Ala Asn Phe Pro Ser Phe
465                 470                 475                 480

Pro Leu Pro His His Lys His Pro Leu Lys Pro Asp Asn Gln Pro Phe
                485                 490                 495

Pro Gln Ser Val Ser Glu Ser Cys Pro Gly Lys Phe Lys Ser Gly Phe
            500                 505                 510

Pro Gln Val Ser Met Phe Phe Thr His Thr Phe Pro Lys
        515                 520                 525
```

<210> 43
<211> 499
<212> PRT
<213> Homo sapiens

<220> misc_feature
<222> (181)..(193)
<223> heparin binding domain

<400> 43

```
Met Asp Thr Ser Pro Leu Cys Phe Ser Ile Leu Leu Val Leu Cys Ile
1               5                   10                  15

Phe Ile Gln Ser Ser Ala Leu Gly Gln Ser Leu Lys Pro Glu Pro Phe
            20                  25                  30

Gly Arg Arg Ala Gln Ala Val Glu Thr Asn Lys Thr Leu His Glu Met
        35                  40                  45

Lys Thr Arg Phe Leu Leu Phe Gly Glu Thr Asn Gln Gly Cys Gln Ile
    50                  55                  60
```

```
Arg Ile Asn His Pro Asp Thr Leu Gln Glu Cys Gly Phe Asn Ser Ser
65                  70                  75                  80

Leu Pro Leu Val Met Ile Ile His Gly Trp Ser Val Asp Gly Val Leu
                85                  90                  95

Glu Asn Trp Ile Trp Gln Met Val Ala Ala Leu Lys Ser Gln Pro Ala
            100                 105                 110

Gln Pro Val Asn Val Gly Leu Val Asp Trp Ile Thr Leu Ala His Asp
        115                 120                 125

His Tyr Thr Ile Ala Val Arg Asn Thr Arg Leu Val Gly Lys Glu Val
    130                 135                 140

Ala Ala Leu Leu Arg Trp Leu Glu Glu Ser Val Gln Leu Ser Arg Ser
145                 150                 155                 160

His Val His Leu Ile Gly Tyr Ser Leu Gly Ala His Val Ser Gly Phe
                165                 170                 175

Ala Gly Ser Ser Ile Gly Gly Thr His Lys Ile Gly Arg Ile Thr Gly
            180                 185                 190

Leu Asp Ala Ala Gly Pro Leu Phe Glu Gly Ser Ala Pro Ser Asn Arg
        195                 200                 205

Leu Ser Pro Asp Asp Ala Asn Phe Val Asp Ala Ile His Thr Phe Thr
    210                 215                 220

Arg Glu His Met Gly Leu Ser Val Gly Ile Lys Gln Pro Ile Gly His
225                 230                 235                 240

Tyr Asp Phe Tyr Pro Asn Gly Gly Ser Phe Gln Pro Gly Cys His Phe
                245                 250                 255

Leu Glu Leu Tyr Arg His Ile Ala Gln His Gly Phe Asn Ala Ile Thr
            260                 265                 270

Gln Thr Ile Lys Cys Ser His Glu Arg Ser Val His Leu Phe Ile Asp
        275                 280                 285

Ser Leu Leu His Ala Gly Thr Gln Ser Met Ala Tyr Pro Cys Gly Asp
    290                 295                 300

Met Asn Ser Phe Ser Gln Gly Leu Cys Leu Ser Cys Lys Lys Gly Arg
305                 310                 315                 320
```

```
Cys Asn Thr Leu Gly Tyr His Val Arg Gln Glu Pro Arg Ser Lys Ser
                325                 330                 335

Lys Arg Leu Phe Leu Val Thr Arg Ala Gln Ser Pro Phe Lys Val Tyr
            340                 345                 350

His Tyr Gln Leu Lys Ile Gln Phe Ile Asn Gln Thr Glu Thr Pro Ile
        355                 360                 365

Gln Thr Thr Phe Thr Met Ser Leu Leu Gly Thr Lys Glu Lys Met Gln
    370                 375                 380

Lys Ile Pro Ile Thr Leu Gly Lys Gly Ile Ala Ser Asn Lys Thr Tyr
385                 390                 395                 400

Ser Phe Leu Ile Thr Leu Asp Val Asp Ile Gly Glu Leu Ile Met Ile
                405                 410                 415

Lys Phe Lys Trp Glu Asn Ser Ala Val Trp Ala Asn Val Trp Asp Thr
            420                 425                 430

Val Gln Thr Ile Ile Pro Trp Ser Thr Gly Pro Arg His Ser Gly Leu
        435                 440                 445

Val Leu Lys Thr Ile Arg Val Lys Ala Gly Glu Thr Gln Gln Arg Met
    450                 455                 460

Thr Phe Cys Ser Glu Asn Thr Asp Asp Leu Leu Leu Arg Pro Thr Gln
465                 470                 475                 480

Glu Lys Ile Phe Val Lys Cys Glu Ile Lys Ser Lys Thr Ser Lys Arg
                485                 490                 495

Lys Ile Arg
```

<210> 44
<211> 475
<212> PRT
<213> Homo sapiens

<220> misc_feature
<222> (319)..(331)
<223> heparin binding domain

<400> 44

```
Met Glu Ser Lys Ala Leu Leu Val Leu Thr Leu Ala Val Trp Leu Gln
1               5                   10                  15
```

Ser Leu Thr Ala Ser Arg Gly Gly Val Ala Ala Ala Asp Gln Arg Arg
20 25 30

Asp Phe Ile Asp Ile Glu Ser Lys Phe Ala Leu Arg Thr Pro Glu Asp
35 40 45

Thr Ala Glu Asp Thr Cys His Leu Ile Pro Gly Val Ala Glu Ser Val
50 55 60

Ala Thr Cys His Phe Asn His Ser Ser Lys Thr Phe Met Val Ile His
65 70 75 80

Gly Trp Thr Val Thr Gly Met Tyr Glu Ser Trp Val Pro Lys Leu Val
85 90 95

Ala Ala Leu Tyr Lys Arg Glu Pro Asp Ser Asn Val Ile Val Val Asp
100 105 110

Trp Leu Ser Arg Ala Gln Glu His Tyr Pro Val Ser Ala Gly Tyr Thr
115 120 125

Lys Leu Val Gly Gln Asp Val Ala Arg Phe Ile Asn Trp Met Glu Glu
130 135 140

Glu Phe Asn Tyr Pro Leu Asp Asn Val His Leu Leu Gly Tyr Ser Leu
145 150 155 160

Gly Ala His Ala Ala Gly Ile Ala Gly Ser Leu Thr Asn Lys Lys Val
165 170 175

Asn Arg Ile Thr Gly Leu Asp Pro Ala Gly Pro Asn Phe Glu Tyr Ala
180 185 190

Glu Ala Pro Ser Arg Leu Ser Pro Asp Asp Ala Asp Phe Val Asp Val
195 200 205

Leu His Thr Phe Thr Arg Gly Ser Pro Gly Arg Ser Ile Gly Ile Gln
210 215 220

Lys Pro Val Gly His Val Asp Ile Tyr Pro Asn Gly Gly Thr Phe Gln
225 230 235 240

Pro Gly Cys Asn Ile Gly Glu Ala Ile Arg Val Ile Ala Glu Arg Gly
245 250 255

Leu Gly Asp Val Asp Gln Leu Val Lys Cys Ser His Glu Arg Ser Ile
260 265 270

```
His Leu Phe Ile Asp Ser Leu Leu Asn Glu Glu Asn Pro Ser Lys Ala
        275                 280                 285

Tyr Arg Cys Ser Ser Lys Glu Ala Phe Glu Lys Gly Leu Cys Leu Ser
    290                 295                 300

Cys Arg Lys Asn Arg Cys Asn Asn Leu Gly Tyr Glu Ile Asn Lys Val
305                 310                 315                 320

Arg Ala Lys Arg Ser Ser Lys Met Tyr Leu Lys Thr Arg Ser Gln Met
                325                 330                 335

Pro Tyr Lys Val Phe His Tyr Gln Val Lys Ile His Phe Ser Gly Thr
            340                 345                 350

Glu Ser Glu Thr His Thr Asn Gln Ala Phe Glu Ile Ser Leu Tyr Gly
        355                 360                 365

Thr Val Ala Glu Ser Glu Asn Ile Pro Phe Thr Leu Pro Glu Val Ser
    370                 375                 380

Thr Asn Lys Thr Tyr Ser Phe Leu Ile Tyr Thr Glu Val Asp Ile Gly
385                 390                 395                 400

Glu Leu Leu Met Leu Lys Leu Lys Trp Lys Ser Asp Ser Tyr Phe Ser
                405                 410                 415

Trp Ser Asp Trp Trp Ser Ser Pro Gly Phe Ala Ile Gln Lys Ile Arg
            420                 425                 430

Val Lys Ala Gly Glu Thr Gln Lys Lys Val Ile Phe Cys Ser Arg Glu
        435                 440                 445

Lys Val Ser His Leu Gln Lys Gly Lys Ala Pro Ala Val Phe Val Lys
    450                 455                 460

Cys His Asp Lys Ser Leu Asn Lys Lys Ser Gly
465                 470                 475
```

<210> 45
<211> 357
<212> PRT
<213> Homo sapiens

<400> 45

```
Met Ala Pro Arg Arg Val Arg Ser Phe Leu Arg Gly Leu Pro Ala Leu
1               5                   10                  15
```

```
Leu Leu Leu Leu Leu Phe Leu Gly Pro Trp Pro Ala Ala Ser His Gly
            20                  25                  30

Gly Lys Tyr Ser Arg Glu Lys Asn Gln Pro Lys Pro Ser Pro Lys Arg
        35                  40                  45

Glu Ser Gly Glu Glu Phe Arg Met Glu Lys Leu Asn Gln Leu Trp Glu
    50                  55                  60

Lys Ala Gln Arg Leu His Leu Pro Pro Val Arg Leu Ala Glu Leu His
65                  70                  75                  80

Ala Asp Leu Lys Ile Gln Glu Arg Asp Glu Leu Ala Trp Lys Lys Leu
                85                  90                  95

Lys Leu Asp Gly Leu Asp Glu Asp Gly Glu Lys Glu Ala Arg Leu Ile
            100                 105                 110

Arg Asn Leu Asn Val Ile Leu Ala Lys Tyr Gly Leu Asp Gly Lys Lys
        115                 120                 125

Asp Ala Arg Gln Val Thr Ser Asn Ser Leu Ser Gly Thr Gln Glu Asp
    130                 135                 140

Gly Leu Asp Asp Pro Arg Leu Glu Lys Leu Trp His Lys Ala Lys Thr
145                 150                 155                 160

Ser Gly Lys Phe Ser Gly Glu Glu Leu Asp Lys Leu Trp Arg Glu Phe
                165                 170                 175

Leu His His Lys Glu Lys Val His Glu Tyr Asn Val Leu Leu Glu Thr
            180                 185                 190

Leu Ser Arg Thr Glu Glu Ile His Glu Asn Val Ile Ser Pro Ser Asp
        195                 200                 205

Leu Ser Asp Ile Lys Gly Ser Val Leu His Ser Arg His Thr Glu Leu
    210                 215                 220

Lys Glu Lys Leu Arg Ser Ile Asn Gln Gly Leu Asp Arg Leu Arg Arg
225                 230                 235                 240

Val Ser His Gln Gly Tyr Ser Thr Glu Ala Glu Phe Glu Glu Pro Arg
                245                 250                 255

Val Ile Asp Leu Trp Asp Leu Ala Gln Ser Ala Asn Leu Thr Asp Lys
            260                 265                 270
```

Glu Leu Glu Ala Phe Arg Glu Glu Leu Lys His Phe Glu Ala Lys Ile
275 280 285

Glu Lys His Asn His Tyr Gln Lys Gln Leu Glu Ile Ala His Glu Lys
290 295 300

Leu Arg His Ala Glu Ser Val Gly Asp Gly Glu Arg Val Ser Arg Ser
305 310 315 320

Arg Glu Lys His Ala Leu Leu Glu Gly Arg Thr Lys Glu Leu Gly Tyr
325 330 335

Thr Val Lys Lys His Leu Gln Asp Leu Ser Gly Arg Ile Ser Arg Ala
340 345 350

Arg His Asn Glu Leu
355

<210> 46
<211> 208
<212> PRT
<213> Homo sapiens

<400> 46

Met Lys Leu Leu Pro Ser Val Val Leu Lys Leu Phe Leu Ala Ala Val
1 5 10 15

Leu Ser Ala Leu Val Thr Gly Glu Ser Leu Glu Arg Leu Arg Arg Gly
20 25 30

Leu Ala Ala Gly Thr Ser Asn Pro Asp Pro Pro Thr Val Ser Thr Asp
35 40 45

Gln Leu Leu Pro Leu Gly Gly Gly Arg Asp Arg Lys Val Arg Asp Leu
50 55 60

Gln Glu Ala Asp Leu Asp Leu Leu Arg Val Thr Leu Ser Ser Lys Pro
65 70 75 80

Gln Ala Leu Ala Thr Pro Asn Lys Glu Glu His Gly Lys Arg Lys Lys
85 90 95

Lys Gly Lys Gly Leu Gly Lys Lys Arg Asp Pro Cys Leu Arg Lys Tyr
100 105 110

Lys Asp Phe Cys Ile His Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg
115 120 125

```
Ala Pro Ser Cys Ile Cys His Pro Gly Tyr His Gly Glu Arg Cys His
    130                 135                 140

Gly Leu Ser Leu Pro Val Glu Asn Arg Leu Tyr Thr Tyr Asp His Thr
145                 150                 155                 160

Thr Ile Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu
                165                 170                 175

Val Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg Arg Gly Gly Tyr
            180                 185                 190

Asp Val Glu Asn Glu Glu Lys Val Lys Leu Gly Met Thr Asn Ser His
        195                 200                 205
```

<210> 47
<211> 221
<212> PRT
<213> Homo sapiens

<220> misc_feature
<222> (193)..(213)
<223> heparin binding domain

<400> 47

```
Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1               5                   10                  15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
            20                  25                  30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
        35                  40                  45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
    50                  55                  60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65                  70                  75                  80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
                85                  90                  95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
            100                 105                 110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
        115                 120                 125
```

```
Glu Cys Arg His Ser Pro Gly Arg Gln Ser Pro Asp Met Pro Gly Asp
    130                 135                 140

Phe Arg Ala Asp Ala Pro Ser Phe Leu Pro Pro Arg Arg Ser Leu Pro
145                 150                 155                 160

Met Leu Phe Arg Met Glu Trp Gly Cys Ala Leu Thr Gly Ser Gln Ser
                165                 170                 175

Ala Val Trp Pro Ser Ser Pro Val Pro Glu Glu Ile Pro Arg Met His
            180                 185                 190

Pro Gly Arg Asn Gly Lys Lys Gln Gln Arg Lys Pro Leu Arg Glu Lys
        195                 200                 205

Met Lys Pro Glu Arg Cys Gly Asp Ala Val Pro Arg Arg
    210                 215                 220
```

<210> 48
<211> 500
<212> PRT
<213> Homo sapiens

<220> misc_feature
<222> (325)..(337)
<223> heparin binding domain

<400> 48

```
Met Ser Asn Ser Val Pro Leu Leu Cys Phe Trp Ser Leu Cys Tyr Cys
1               5                   10                  15

Phe Ala Ala Gly Ser Pro Val Pro Phe Gly Pro Glu Gly Arg Leu Glu
            20                  25                  30

Asp Lys Leu His Lys Pro Lys Ala Thr Gln Thr Glu Val Lys Pro Ser
        35                  40                  45

Val Arg Phe Asn Leu Arg Thr Ser Lys Asp Pro Glu His Glu Gly Cys
    50                  55                  60

Tyr Leu Ser Val Gly His Ser Gln Pro Leu Glu Asp Cys Ser Phe Asn
65                  70                  75                  80

Met Thr Ala Lys Thr Phe Phe Ile Ile His Gly Trp Thr Met Ser Gly
                85                  90                  95

Ile Phe Glu Asn Trp Leu His Lys Leu Val Ser Ala Leu His Thr Arg
            100                 105                 110
```

```
Glu Lys Asp Ala Asn Val Val Val Val Asp Trp Leu Pro Leu Ala His
        115                 120                 125

Gln Leu Tyr Thr Asp Ala Val Asn Asn Thr Arg Val Val Gly His Ser
    130                 135                 140

Ile Ala Arg Met Leu Asp Trp Leu Gln Glu Lys Asp Asp Phe Ser Leu
145                 150                 155                 160

Gly Asn Val His Leu Ile Gly Tyr Ser Leu Gly Ala His Val Ala Gly
                165                 170                 175

Tyr Ala Gly Asn Phe Val Lys Gly Thr Val Gly Arg Ile Thr Gly Leu
            180                 185                 190

Asp Pro Ala Gly Pro Met Phe Glu Gly Ala Asp Ile His Lys Arg Leu
        195                 200                 205

Ser Pro Asp Asp Ala Asp Phe Val Asp Val Leu His Thr Tyr Thr Arg
    210                 215                 220

Ser Phe Gly Leu Ser Ile Gly Ile Gln Met Pro Val Gly His Ile Asp
225                 230                 235                 240

Ile Tyr Pro Asn Gly Gly Asp Phe Gln Pro Gly Cys Gly Leu Asn Asp
                245                 250                 255

Val Leu Gly Ser Ile Ala Tyr Gly Thr Ile Thr Glu Val Val Lys Cys
            260                 265                 270

Glu His Glu Arg Ala Val His Leu Phe Val Asp Ser Leu Val Asn Gln
        275                 280                 285

Asp Lys Pro Ser Phe Ala Phe Gln Cys Thr Asp Ser Asn Arg Phe Lys
    290                 295                 300

Lys Gly Ile Cys Leu Ser Cys Arg Lys Asn Arg Cys Asn Ser Ile Gly
305                 310                 315                 320

Tyr Asn Ala Lys Lys Met Arg Asn Lys Arg Asn Ser Lys Met Tyr Leu
                325                 330                 335

Lys Thr Arg Ala Gly Met Pro Phe Arg Val Tyr His Tyr Gln Met Lys
            340                 345                 350

Ile His Val Phe Ser Tyr Lys Asn Met Gly Glu Ile Glu Pro Thr Phe
        355                 360                 365
```

Tyr Val Thr Leu Tyr Gly Thr Asn Ala Asp Ser Gln Thr Leu Pro Leu
370 375 380

Glu Ile Val Glu Arg Ile Glu Gln Asn Ala Thr Asn Thr Phe Leu Val
385 390 395 400

Tyr Thr Glu Glu Asp Leu Gly Asp Leu Leu Lys Ile Gln Leu Thr Trp
405 410 415

Glu Gly Ala Ser Gln Ser Trp Tyr Asn Leu Trp Lys Glu Phe Arg Ser
420 425 430

Tyr Leu Ser Gln Pro Arg Asn Pro Gly Arg Glu Leu Asn Ile Arg Arg
435 440 445

Ile Arg Val Lys Ser Gly Glu Thr Gln Arg Lys Leu Thr Phe Cys Thr
450 455 460

Glu Asp Pro Glu Asn Thr Ser Ile Ser Pro Gly Arg Glu Leu Trp Phe
465 470 475 480

Arg Lys Cys Arg Asp Gly Trp Arg Met Lys Asn Glu Thr Ser Pro Thr
485 490 495

Val Glu Leu Pro
500

<210> 49
<211> 128
<212> PRT
<213> Homo sapiens

<400> 49

Met Ala Pro Val Lys Lys Leu Val Val Lys Gly Gly Lys Lys Lys Lys
1 5 10 15

Gln Val Leu Lys Phe Thr Leu Asp Cys Thr His Pro Val Glu Asp Gly
20 25 30

Ile Met Asp Ala Ala Asn Phe Glu Gln Phe Leu Gln Glu Arg Ile Lys
35 40 45

Val Asn Gly Lys Ala Gly Asn Leu Gly Gly Gly Val Val Thr Ile Glu
50 55 60

Arg Ser Lys Ser Lys Ile Thr Val Thr Ser Glu Val Pro Phe Ser Lys
65 70 75 80

```
Arg Tyr Leu Lys Tyr Leu Thr Lys Lys Tyr Leu Lys Lys Asn Asn Leu
                85                  90                  95

Arg Asp Trp Leu Arg Val Val Ala Asn Ser Lys Glu Ser Tyr Glu Leu
            100                 105                 110

Arg Tyr Phe Gln Ile Asn Gln Asp Glu Glu Glu Glu Glu Asp Glu Asp
        115                 120                 125
```

<210> 50
<211> 208
<212> PRT
<213> Homo sapiens

<400> 50

```
Met Trp Lys Trp Ile Leu Thr His Cys Ala Ser Ala Phe Pro His Leu
1               5                   10                  15

Pro Gly Cys Cys Cys Cys Cys Phe Leu Leu Leu Phe Leu Val Ser Ser
            20                  25                  30

Val Pro Val Thr Cys Gln Ala Leu Gly Gln Asp Met Val Ser Pro Glu
        35                  40                  45

Ala Thr Asn Ser Ser Ser Ser Ser Phe Ser Ser Pro Ser Ser Ala Gly
    50                  55                  60

Arg His Val Arg Ser Tyr Asn His Leu Gln Gly Asp Val Arg Trp Arg
65                  70                  75                  80

Lys Leu Phe Ser Phe Thr Lys Tyr Phe Leu Lys Ile Glu Lys Asn Gly
                85                  90                  95

Lys Val Ser Gly Thr Lys Lys Glu Asn Cys Pro Tyr Ser Ile Leu Glu
            100                 105                 110

Ile Thr Ser Val Glu Ile Gly Val Val Ala Val Lys Ala Ile Asn Ser
        115                 120                 125

Asn Tyr Tyr Leu Ala Met Asn Lys Lys Gly Lys Leu Tyr Gly Ser Lys
    130                 135                 140

Glu Phe Asn Asn Asp Cys Lys Leu Lys Glu Arg Ile Glu Glu Asn Gly
145                 150                 155                 160

Tyr Asn Thr Tyr Ala Ser Phe Asn Trp Gln His Asn Gly Arg Gln Met
                165                 170                 175
```

```
Tyr Val Ala Leu Asn Gly Lys Gly Ala Pro Arg Arg Gly Gln Lys Thr
            180                 185                 190

Arg Arg Lys Asn Thr Ser Ala His Phe Leu Pro Met Val Val His Ser
        195                 200                 205
```

<210> 51
<211> 208
<212> PRT
<213> Homo sapiens

<400> 51

```
Met Lys Leu Leu Pro Ser Val Val Leu Lys Leu Phe Leu Ala Ala Val
1               5                   10                  15

Leu Ser Ala Leu Val Thr Gly Glu Ser Leu Glu Arg Leu Arg Arg Gly
            20                  25                  30

Leu Ala Ala Gly Thr Ser Asn Pro Asp Pro Pro Thr Val Ser Thr Asp
        35                  40                  45

Gln Leu Leu Pro Leu Gly Gly Gly Arg Asp Arg Lys Val Arg Asp Leu
    50                  55                  60

Gln Glu Ala Asp Leu Asp Leu Leu Arg Val Thr Leu Ser Ser Lys Pro
65                  70                  75                  80

Gln Ala Leu Ala Thr Pro Asn Lys Glu Glu His Gly Lys Arg Lys Lys
                85                  90                  95

Lys Gly Lys Gly Leu Gly Lys Lys Arg Asp Pro Cys Leu Arg Lys Tyr
            100                 105                 110

Lys Asp Phe Cys Ile His Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg
        115                 120                 125

Ala Pro Ser Cys Ile Cys His Pro Gly Tyr His Gly Glu Arg Cys His
    130                 135                 140

Gly Leu Ser Leu Pro Val Glu Asn Arg Leu Tyr Thr Tyr Asp His Thr
145                 150                 155                 160

Thr Ile Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu
                165                 170                 175

Val Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg Arg Gly Gly Tyr
            180                 185                 190
```

Asp Val Glu Asn Glu Glu Lys Val Lys Leu Gly Met Thr Asn Ser His
195 200 205

<210> 52
<211> 1231
<212> PRT
<213> Homo sapiens

<400> 52

Met Arg Leu Leu Ala Lys Ile Ile Cys Leu Met Leu Trp Ala Ile Cys
1 5 10 15

Val Ala Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile
20 25 30

Leu Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala
35 40 45

Ile Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met
50 55 60

Val Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys
65 70 75 80

Gln Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe
85 90 95

Thr Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr
100 105 110

Thr Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu
115 120 125

Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val
130 135 140

Lys Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser
145 150 155 160

Ala Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe
165 170 175

Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys
180 185 190

Ser Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile
195 200 205

```
Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys
    210                 215                 220

Ile Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly
225                 230                 235                 240

Tyr Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp
                245                 250                 255

Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile
            260                 265                 270

Pro Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp
        275                 280                 285

Glu Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly
    290                 295                 300

Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys
305                 310                 315                 320

Thr Leu Lys Pro Cys Asp Tyr Pro Asp Ile Lys His Gly Gly Leu Tyr
                325                 330                 335

His Glu Asn Met Arg Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr
            340                 345                 350

Tyr Ser Tyr Tyr Cys Asp Glu His Phe Glu Thr Pro Ser Gly Ser Tyr
        355                 360                 365

Trp Asp His Ile His Cys Thr Gln Asp Gly Trp Ser Pro Ala Val Pro
    370                 375                 380

Cys Leu Arg Lys Cys Tyr Phe Pro Tyr Leu Glu Asn Gly Tyr Asn Gln
385                 390                 395                 400

Asn His Gly Arg Lys Phe Val Gln Gly Lys Ser Ile Asp Val Ala Cys
                405                 410                 415

His Pro Gly Tyr Ala Leu Pro Lys Ala Gln Thr Thr Val Thr Cys Met
            420                 425                 430

Glu Asn Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Val Lys Thr Cys
        435                 440                 445

Ser Lys Ser Ser Ile Asp Ile Glu Asn Gly Phe Ile Ser Glu Ser Gln
    450                 455                 460
```

```
Tyr Thr Tyr Ala Leu Lys Glu Lys Ala Lys Tyr Gln Cys Lys Leu Gly
465                 470                 475                 480

Tyr Val Thr Ala Asp Gly Glu Thr Ser Gly Ser Ile Arg Cys Gly Lys
                485                 490                 495

Asp Gly Trp Ser Ala Gln Pro Thr Cys Ile Lys Ser Cys Asp Ile Pro
            500                 505                 510

Val Phe Met Asn Ala Arg Thr Lys Asn Asp Phe Thr Trp Phe Lys Leu
        515                 520                 525

Asn Asp Thr Leu Asp Tyr Glu Cys His Asp Gly Tyr Glu Ser Asn Thr
    530                 535                 540

Gly Ser Thr Thr Gly Ser Ile Val Cys Gly Tyr Asn Gly Trp Ser Asp
545                 550                 555                 560

Leu Pro Ile Cys Tyr Glu Arg Glu Cys Glu Leu Pro Lys Ile Asp Val
                565                 570                 575

His Leu Val Pro Asp Arg Lys Lys Asp Gln Tyr Lys Val Gly Glu Val
            580                 585                 590

Leu Lys Phe Ser Cys Lys Pro Gly Phe Thr Ile Val Gly Pro Asn Ser
        595                 600                 605

Val Gln Cys Tyr His Phe Gly Leu Ser Pro Asp Leu Pro Ile Cys Lys
    610                 615                 620

Glu Gln Val Gln Ser Cys Gly Pro Pro Pro Glu Leu Leu Asn Gly Asn
625                 630                 635                 640

Val Lys Glu Lys Thr Lys Glu Glu Tyr Gly His Ser Glu Val Val Glu
                645                 650                 655

Tyr Tyr Cys Asn Pro Arg Phe Leu Met Lys Gly Pro Asn Lys Ile Gln
            660                 665                 670

Cys Val Asp Gly Glu Trp Thr Thr Leu Pro Val Cys Ile Val Glu Glu
        675                 680                 685

Ser Thr Cys Gly Asp Ile Pro Glu Leu Glu His Gly Trp Ala Gln Leu
    690                 695                 700

Ser Ser Pro Pro Tyr Tyr Tyr Gly Asp Ser Val Glu Phe Asn Cys Ser
705                 710                 715                 720
```

```
Glu Ser Phe Thr Met Ile Gly His Arg Ser Ile Thr Cys Ile His Gly
                725                 730                 735

Val Trp Thr Gln Leu Pro Gln Cys Val Ala Ile Asp Lys Leu Lys Lys
            740                 745                 750

Cys Lys Ser Ser Asn Leu Ile Ile Leu Glu Glu His Leu Lys Asn Lys
        755                 760                 765

Lys Glu Phe Asp His Asn Ser Asn Ile Arg Tyr Arg Cys Arg Gly Lys
    770                 775                 780

Glu Gly Trp Ile His Thr Val Cys Ile Asn Gly Arg Trp Asp Pro Glu
785                 790                 795                 800

Val Asn Cys Ser Met Ala Gln Ile Gln Leu Cys Pro Pro Pro Pro Gln
                805                 810                 815

Ile Pro Asn Ser His Asn Met Thr Thr Thr Leu Asn Tyr Arg Asp Gly
            820                 825                 830

Glu Lys Val Ser Val Leu Cys Gln Glu Asn Tyr Leu Ile Gln Glu Gly
        835                 840                 845

Glu Glu Ile Thr Cys Lys Asp Gly Arg Trp Gln Ser Ile Pro Leu Cys
    850                 855                 860

Val Glu Lys Ile Pro Cys Ser Gln Pro Pro Gln Ile Glu His Gly Thr
865                 870                 875                 880

Ile Asn Ser Ser Arg Ser Ser Gln Glu Ser Tyr Ala His Gly Thr Lys
                885                 890                 895

Leu Ser Tyr Thr Cys Glu Gly Gly Phe Arg Ile Ser Glu Glu Asn Glu
            900                 905                 910

Thr Thr Cys Tyr Met Gly Lys Trp Ser Ser Pro Pro Gln Cys Glu Gly
        915                 920                 925

Leu Pro Cys Lys Ser Pro Pro Glu Ile Ser His Gly Val Val Ala His
    930                 935                 940

Met Ser Asp Ser Tyr Gln Tyr Gly Glu Glu Val Thr Tyr Lys Cys Phe
945                 950                 955                 960

Glu Gly Phe Gly Ile Asp Gly Pro Ala Ile Ala Lys Cys Leu Gly Glu
                965                 970                 975
```

```
Lys Trp Ser His Pro Pro Ser Cys Ile Lys Thr Asp Cys Leu Ser Leu
          980                 985                 990

Pro Ser Phe Glu Asn Ala Ile Pro  Met Gly Glu Lys Lys  Asp Val Tyr
       995                1000                1005

Lys Ala  Gly Glu Gln Val Thr  Tyr Thr Cys Ala Thr  Tyr Tyr Lys
    1010                1015                1020

Met Asp  Gly Ala Ser Asn Val  Thr Cys Ile Asn Ser  Arg Trp Thr
    1025                1030                1035

Gly Arg  Pro Thr Cys Arg Asp  Thr Ser Cys Val Asn  Pro Pro Thr
    1040                1045                1050

Val Gln  Asn Ala Tyr Ile Val  Ser Arg Gln Met Ser  Lys Tyr Pro
    1055                1060                1065

Ser Gly  Glu Arg Val Arg Tyr  Gln Cys Arg Ser Pro  Tyr Glu Met
    1070                1075                1080

Phe Gly  Asp Glu Glu Val Met  Cys Leu Asn Gly Asn  Trp Thr Glu
    1085                1090                1095

Pro Pro  Gln Cys Lys Asp Ser  Thr Gly Lys Cys Gly  Pro Pro Pro
    1100                1105                1110

Pro Ile  Asp Asn Gly Asp Ile  Thr Ser Phe Pro Leu  Ser Val Tyr
    1115                1120                1125

Ala Pro  Ala Ser Ser Val Glu  Tyr Gln Cys Gln Asn  Leu Tyr Gln
    1130                1135                1140

Leu Glu  Gly Asn Lys Arg Ile  Thr Cys Arg Asn Gly  Gln Trp Ser
    1145                1150                1155

Glu Pro  Pro Lys Cys Leu His  Pro Cys Val Ile Ser  Arg Glu Ile
    1160                1165                1170

Met Glu  Asn Tyr Asn Ile Ala  Leu Arg Trp Thr Ala  Lys Gln Lys
    1175                1180                1185

Leu Tyr  Ser Arg Thr Gly Glu  Ser Val Glu Phe Val  Cys Lys Arg
    1190                1195                1200

Gly Tyr  Arg Leu Ser Ser Arg  Ser His Thr Leu Arg  Thr Thr Cys
    1205                1210                1215
```

```
Trp Asp  Gly Lys Leu Glu Tyr  Pro Thr Cys Ala Lys  Arg
    1220                 1225                 1230


<210>  53
<211>  357
<212>  PRT
<213>  Homo sapiens

<400>  53

Met Ala Pro Arg Arg Val Arg Ser Phe Leu Arg Gly Leu Pro Ala Leu
1               5                  10                  15


Leu Leu Leu Leu Leu Phe Leu Gly Pro Trp Pro Ala Ala Ser His Gly
            20                  25                  30


Gly Lys Tyr Ser Arg Glu Lys Asn Gln Pro Lys Pro Ser Pro Lys Arg
        35                  40                  45


Glu Ser Gly Glu Glu Phe Arg Met Glu Lys Leu Asn Gln Leu Trp Glu
    50                  55                  60


Lys Ala Gln Arg Leu His Leu Pro Pro Val Arg Leu Ala Glu Leu His
65                  70                  75                  80


Ala Asp Leu Lys Ile Gln Glu Arg Asp Glu Leu Ala Trp Lys Lys Leu
                85                  90                  95


Lys Leu Asp Gly Leu Asp Glu Asp Gly Glu Lys Glu Ala Arg Leu Ile
            100                 105                 110


Arg Asn Leu Asn Val Ile Leu Ala Lys Tyr Gly Leu Asp Gly Lys Lys
        115                 120                 125


Asp Ala Arg Gln Val Thr Ser Asn Ser Leu Ser Gly Thr Gln Glu Asp
    130                 135                 140


Gly Leu Asp Asp Pro Arg Leu Glu Lys Leu Trp His Lys Ala Lys Thr
145                 150                 155                 160


Ser Gly Lys Phe Ser Gly Glu Glu Leu Asp Lys Leu Trp Arg Glu Phe
                165                 170                 175


Leu His His Lys Glu Lys Val His Glu Tyr Asn Val Leu Leu Glu Thr
            180                 185                 190


Leu Ser Arg Thr Glu Glu Ile His Glu Asn Val Ile Ser Pro Ser Asp
        195                 200                 205
```

```
Leu Ser Asp Ile Lys Gly Ser Val Leu His Ser Arg His Thr Glu Leu
    210                 215                 220

Lys Glu Lys Leu Arg Ser Ile Asn Gln Gly Leu Asp Arg Leu Arg Arg
225                 230                 235                 240

Val Ser His Gln Gly Tyr Ser Thr Glu Ala Glu Phe Glu Glu Pro Arg
            245                 250                 255

Val Ile Asp Leu Trp Asp Leu Ala Gln Ser Ala Asn Leu Thr Asp Lys
        260                 265                 270

Glu Leu Glu Ala Phe Arg Glu Glu Leu Lys His Phe Glu Ala Lys Ile
    275                 280                 285

Glu Lys His Asn His Tyr Gln Lys Gln Leu Glu Ile Ala His Glu Lys
    290                 295                 300

Leu Arg His Ala Glu Ser Val Gly Asp Gly Glu Arg Val Ser Arg Ser
305                 310                 315                 320

Arg Glu Lys His Ala Leu Leu Glu Gly Arg Thr Lys Glu Leu Gly Tyr
            325                 330                 335

Thr Val Lys Lys His Leu Gln Asp Leu Ser Gly Arg Ile Ser Arg Ala
        340                 345                 350

Arg His Asn Glu Leu
    355
```

<210> 54
<211> 317
<212> PRT
<213> Homo sapiens

<400> 54

```
Met Lys Val Leu Trp Ala Ala Leu Leu Val Thr Phe Leu Ala Gly Cys
1               5                   10                  15

Gln Ala Lys Val Glu Gln Ala Val Glu Thr Glu Pro Glu Pro Glu Leu
            20                  25                  30

Arg Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu
        35                  40                  45

Gly Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln
    50                  55                  60
```

```
Val Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala
65                  70                  75                  80

Leu Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu
                85                  90                  95

Glu Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser
            100                 105                 110

Lys Glu Leu Gln Ala Ala Gln Ala Arg Leu Gly Ala Asp Met Glu Asp
        115                 120                 125

Val Cys Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu
    130                 135                 140

Gly Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg
145                 150                 155                 160

Lys Leu Arg Lys Arg Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg
                165                 170                 175

Leu Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu
            180                 185                 190

Ser Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val
        195                 200                 205

Arg Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg
    210                 215                 220

Ala Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly
225                 230                 235                 240

Ser Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu
                245                 250                 255

Val Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala
            260                 265                 270

Glu Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu
        275                 280                 285

Asp Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala
    290                 295                 300

Val Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
305                 310                 315
```

<210> 55
<211> 1404
<212> PRT
<213> Homo sapiens

<400> 55

Met Ala Trp Lys Thr Leu Pro Ile Tyr Leu Leu Leu Leu Leu Ser Val
1 5 10 15

Phe Val Ile Gln Gln Val Ser Ser Gln Asp Leu Ser Ser Cys Ala Gly
20 25 30

Arg Cys Gly Glu Gly Tyr Ser Arg Asp Ala Thr Cys Asn Cys Asp Tyr
35 40 45

Asn Cys Gln His Tyr Met Glu Cys Cys Pro Asp Phe Lys Arg Val Cys
50 55 60

Thr Ala Glu Leu Ser Cys Lys Gly Arg Cys Phe Glu Ser Phe Glu Arg
65 70 75 80

Gly Arg Glu Cys Asp Cys Asp Ala Gln Cys Lys Lys Tyr Asp Lys Cys
85 90 95

Cys Pro Asp Tyr Glu Ser Phe Cys Ala Glu Val His Asn Pro Thr Ser
100 105 110

Pro Pro Ser Ser Lys Lys Ala Pro Pro Pro Ser Gly Ala Ser Gln Thr
115 120 125

Ile Lys Ser Thr Thr Lys Arg Ser Pro Lys Pro Pro Asn Lys Lys Lys
130 135 140

Thr Lys Lys Val Ile Glu Ser Glu Glu Ile Thr Glu Glu His Ser Val
145 150 155 160

Ser Glu Asn Gln Glu Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser
165 170 175

Ser Thr Ile Trp Lys Ile Lys Ser Ser Lys Asn Ser Ala Ala Asn Arg
180 185 190

Glu Leu Gln Lys Lys Leu Lys Val Lys Asp Asn Lys Lys Asn Arg Thr
195 200 205

Lys Lys Lys Pro Thr Pro Lys Pro Pro Val Val Asp Glu Ala Gly Ser
210 215 220

```
Gly Leu Asp Asn Gly Asp Phe Lys Val Thr Thr Pro Asp Thr Ser Thr
225                 230                 235                 240

Thr Gln His Asn Lys Val Ser Thr Ser Pro Lys Ile Thr Thr Ala Lys
                245                 250                 255

Pro Ile Asn Pro Arg Pro Ser Leu Pro Pro Asn Ser Asp Thr Ser Lys
            260                 265                 270

Glu Thr Ser Leu Thr Val Asn Lys Glu Thr Thr Val Glu Thr Lys Glu
        275                 280                 285

Thr Thr Thr Thr Asn Lys Gln Thr Ser Thr Asp Gly Lys Glu Lys Thr
    290                 295                 300

Thr Ser Ala Lys Glu Thr Gln Ser Ile Glu Lys Thr Ser Ala Lys Asp
305                 310                 315                 320

Leu Ala Pro Thr Ser Lys Val Leu Ala Lys Pro Thr Pro Lys Ala Glu
                325                 330                 335

Thr Thr Thr Lys Gly Pro Ala Leu Thr Thr Pro Lys Glu Pro Thr Pro
            340                 345                 350

Thr Thr Pro Lys Glu Pro Ala Ser Thr Thr Pro Lys Glu Pro Thr Pro
        355                 360                 365

Thr Thr Ile Lys Ser Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr
    370                 375                 380

Thr Thr Lys Ser Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr Thr
385                 390                 395                 400

Thr Lys Glu Pro Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr Thr
                405                 410                 415

Thr Lys Glu Pro Ala Pro Thr Thr Thr Lys Ser Ala Pro Thr Thr Pro
            420                 425                 430

Lys Glu Pro Ala Pro Thr Thr Pro Lys Lys Pro Ala Pro Thr Thr Pro
        435                 440                 445

Lys Glu Pro Ala Pro Thr Thr Pro Lys Glu Pro Thr Pro Thr Thr Pro
    450                 455                 460

Lys Glu Pro Ala Pro Thr Thr Lys Glu Pro Ala Pro Thr Thr Pro Lys
465                 470                 475                 480
```

Glu Pro Ala Pro Thr Ala Pro Lys Lys Pro Ala Pro Thr Thr Pro Lys
485 490 495

Glu Pro Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr Thr Thr Lys
500 505 510

Glu Pro Ser Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr Thr Thr Lys
515 520 525

Ser Ala Pro Thr Thr Thr Lys Glu Pro Ala Pro Thr Thr Thr Lys Ser
530 535 540

Ala Pro Thr Thr Pro Lys Glu Pro Ser Pro Thr Thr Thr Lys Glu Pro
545 550 555 560

Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr Thr Pro Lys Lys Pro
565 570 575

Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr Thr Pro Lys Glu Pro
580 585 590

Ala Pro Thr Thr Thr Lys Lys Pro Ala Pro Thr Ala Pro Lys Glu Pro
595 600 605

Ala Pro Thr Thr Pro Lys Glu Thr Ala Pro Thr Thr Pro Lys Lys Leu
610 615 620

Thr Pro Thr Thr Pro Glu Lys Leu Ala Pro Thr Thr Pro Glu Lys Pro
625 630 635 640

Ala Pro Thr Thr Pro Glu Glu Leu Ala Pro Thr Thr Pro Glu Glu Pro
645 650 655

Thr Pro Thr Thr Pro Glu Glu Pro Ala Pro Thr Thr Pro Lys Ala Ala
660 665 670

Ala Pro Asn Thr Pro Lys Glu Pro Ala Pro Thr Thr Pro Lys Glu Pro
675 680 685

Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr Thr Pro Lys Glu Thr
690 695 700

Ala Pro Thr Thr Pro Lys Gly Thr Ala Pro Thr Thr Leu Lys Glu Pro
705 710 715 720

Ala Pro Thr Thr Pro Lys Lys Pro Ala Pro Lys Glu Leu Ala Pro Thr
725 730 735

```
Thr Thr Lys Glu Pro Thr Ser Thr Thr Ser Asp Lys Pro Ala Pro Thr
            740                 745                 750

Thr Pro Lys Gly Thr Ala Pro Thr Thr Pro Lys Glu Pro Ala Pro Thr
        755                 760                 765

Thr Pro Lys Glu Pro Ala Pro Thr Thr Pro Lys Gly Thr Ala Pro Thr
    770                 775                 780

Thr Leu Lys Glu Pro Ala Pro Thr Thr Pro Lys Lys Pro Ala Pro Lys
785                 790                 795                 800

Glu Leu Ala Pro Thr Thr Thr Lys Gly Pro Thr Ser Thr Thr Ser Asp
                805                 810                 815

Lys Pro Ala Pro Thr Thr Pro Lys Glu Thr Ala Pro Thr Thr Pro Lys
            820                 825                 830

Glu Pro Ala Pro Thr Thr Pro Lys Lys Pro Ala Pro Thr Thr Pro Glu
        835                 840                 845

Thr Pro Pro Pro Thr Thr Ser Glu Val Ser Thr Pro Thr Thr Thr Lys
    850                 855                 860

Glu Pro Thr Thr Ile His Lys Ser Pro Asp Glu Ser Thr Pro Glu Leu
865                 870                 875                 880

Ser Ala Glu Pro Thr Pro Lys Ala Leu Glu Asn Ser Pro Lys Glu Pro
                885                 890                 895

Gly Val Pro Thr Thr Lys Thr Pro Ala Ala Thr Lys Pro Glu Met Thr
            900                 905                 910

Thr Thr Ala Lys Asp Lys Thr Thr Glu Arg Asp Leu Arg Thr Thr Pro
        915                 920                 925

Glu Thr Thr Thr Ala Ala Pro Lys Met Thr Lys Glu Thr Ala Thr Thr
    930                 935                 940

Thr Glu Lys Thr Thr Glu Ser Lys Ile Thr Ala Thr Thr Thr Gln Val
945                 950                 955                 960

Thr Ser Thr Thr Thr Gln Asp Thr Thr Pro Phe Lys Ile Thr Thr Leu
                965                 970                 975

Lys Thr Thr Thr Leu Ala Pro Lys Val Thr Thr Thr Lys Lys Thr Ile
            980                 985                 990
```

```
Thr Thr Thr Glu Ile Met Asn Lys  Pro Glu Glu Thr Ala  Lys Pro Lys
        995                 1000                1005

Asp Arg  Ala Thr Asn Ser Lys  Ala Thr Thr Pro Lys  Pro Gln Lys
    1010                1015                1020

Pro Thr  Lys Ala Pro Lys Lys  Pro Thr Ser Thr Lys  Lys Pro Lys
    1025                1030                1035

Thr Met  Pro Arg Val Arg Lys  Pro Lys Thr Thr Pro  Thr Pro Arg
    1040                1045                1050

Lys Met  Thr Ser Thr Met Pro  Glu Leu Asn Pro Thr  Ser Arg Ile
    1055                1060                1065

Ala Glu  Ala Met Leu Gln Thr  Thr Thr Arg Pro Asn  Gln Thr Pro
    1070                1075                1080

Asn Ser  Lys Leu Val Glu Val  Asn Pro Lys Ser Glu  Asp Ala Gly
    1085                1090                1095

Gly Ala  Glu Gly Glu Thr Pro  His Met Leu Leu Arg  Pro His Val
    1100                1105                1110

Phe Met  Pro Glu Val Thr Pro  Asp Met Asp Tyr Leu  Pro Arg Val
    1115                1120                1125

Pro Asn  Gln Gly Ile Ile Ile  Asn Pro Met Leu Ser  Asp Glu Thr
    1130                1135                1140

Asn Ile  Cys Asn Gly Lys Pro  Val Asp Gly Leu Thr  Thr Leu Arg
    1145                1150                1155

Asn Gly  Thr Leu Val Ala Phe  Arg Gly His Tyr Phe  Trp Met Leu
    1160                1165                1170

Ser Pro  Phe Ser Pro Pro Ser  Pro Ala Arg Arg Ile  Thr Glu Val
    1175                1180                1185

Trp Gly  Ile Pro Ser Pro Ile  Asp Thr Val Phe Thr  Arg Cys Asn
    1190                1195                1200

Cys Glu  Gly Lys Thr Phe Phe  Phe Lys Asp Ser Gln  Tyr Trp Arg
    1205                1210                1215

Phe Thr  Asn Asp Ile Lys Asp  Ala Gly Tyr Pro Lys  Pro Ile Phe
    1220                1225                1230
```

```
Lys Gly  Phe Gly Gly Leu Thr  Gly Gln Ile Val Ala  Ala Leu Ser
    1235                 1240                 1245

Thr Ala  Lys Tyr Lys Asn Trp  Pro Glu Ser Val Tyr  Phe Phe Lys
    1250                 1255                 1260

Arg Gly  Gly Ser Ile Gln Gln  Tyr Ile Tyr Lys Gln  Glu Pro Val
    1265                 1270                 1275

Gln Lys  Cys Pro Gly Arg Arg  Pro Ala Leu Asn Tyr  Pro Val Tyr
    1280                 1285                 1290

Gly Glu  Met Thr Gln Val Arg  Arg Arg Arg Phe Glu  Arg Ala Ile
    1295                 1300                 1305

Gly Pro  Ser Gln Thr His Thr  Ile Arg Ile Gln Tyr  Ser Pro Ala
    1310                 1315                 1320

Arg Leu  Ala Tyr Gln Asp Lys  Gly Val Leu His Asn  Glu Val Lys
    1325                 1330                 1335

Val Ser  Ile Leu Trp Arg Gly  Leu Pro Asn Val Val  Thr Ser Ala
    1340                 1345                 1350

Ile Ser  Leu Pro Asn Ile Arg  Lys Pro Asp Gly Tyr  Asp Tyr Tyr
    1355                 1360                 1365

Ala Phe  Ser Lys Asp Gln Tyr  Tyr Asn Ile Asp Val  Pro Ser Arg
    1370                 1375                 1380

Thr Ala  Arg Ala Ile Thr Thr  Arg Ser Gly Gln Thr  Leu Ser Lys
    1385                 1390                 1395

Val Trp  Tyr Asn Cys Pro
    1400
```

<210> 56
<211> 499
<212> PRT
<213> Homo sapiens

<400> 56

```
Met Lys His Ser Leu Asn Ala Leu Leu Ile Phe Leu Ile Ile Thr Ser
1               5                  10                  15

Ala Trp Gly Gly Ser Lys Gly Pro Leu Asp Gln Leu Glu Lys Gly Gly
            20                  25                  30
```

```
Glu Thr Ala Gln Ser Ala Asp Pro Gln Trp Glu Gln Leu Asn Asn Lys
        35                  40                  45

Asn Leu Ser Met Pro Leu Leu Pro Ala Asp Phe His Lys Glu Asn Thr
    50                  55                  60

Val Thr Asn Asp Trp Ile Pro Glu Gly Glu Glu Asp Asp Asp Tyr Leu
65                  70                  75                  80

Asp Leu Glu Lys Ile Phe Ser Glu Asp Asp Asp Tyr Ile Asp Ile Val
                85                  90                  95

Asp Ser Leu Ser Val Ser Pro Thr Asp Ser Asp Val Ser Ala Gly Asn
            100                 105                 110

Ile Leu Gln Leu Phe His Gly Lys Ser Arg Ile Gln Arg Leu Asn Ile
        115                 120                 125

Leu Asn Ala Lys Phe Ala Phe Asn Leu Tyr Arg Val Leu Lys Asp Gln
    130                 135                 140

Val Asn Thr Phe Asp Asn Ile Phe Ile Ala Pro Val Gly Ile Ser Thr
145                 150                 155                 160

Ala Met Gly Met Ile Ser Leu Gly Leu Lys Gly Glu Thr His Glu Gln
                165                 170                 175

Val His Ser Ile Leu His Phe Lys Asp Phe Val Asn Ala Ser Ser Lys
            180                 185                 190

Tyr Glu Ile Thr Thr Ile His Asn Leu Phe Arg Lys Leu Thr His Arg
        195                 200                 205

Leu Phe Arg Arg Asn Phe Gly Tyr Thr Leu Arg Ser Val Asn Asp Leu
    210                 215                 220

Tyr Ile Gln Lys Gln Phe Pro Ile Leu Leu Asp Phe Lys Thr Lys Val
225                 230                 235                 240

Arg Glu Tyr Tyr Phe Ala Glu Ala Gln Ile Ala Asp Phe Ser Asp Pro
                245                 250                 255

Ala Phe Ile Ser Lys Thr Asn Asn His Ile Met Lys Leu Thr Lys Gly
            260                 265                 270

Leu Ile Lys Asp Ala Leu Glu Asn Ile Asp Pro Ala Thr Gln Met Met
        275                 280                 285
```

```
Ile Leu Asn Cys Ile Tyr Phe Lys Gly Ser Trp Val Asn Lys Phe Pro
    290                 295                 300

Val Glu Met Thr His Asn His Asn Phe Arg Leu Asn Glu Arg Glu Val
305                 310                 315                 320

Val Lys Val Ser Met Met Gln Thr Lys Gly Asn Phe Leu Ala Ala Asn
                325                 330                 335

Asp Gln Glu Leu Asp Cys Asp Ile Leu Gln Leu Glu Tyr Val Gly Gly
            340                 345                 350

Ile Ser Met Leu Ile Val Val Pro His Lys Met Ser Gly Met Lys Thr
        355                 360                 365

Leu Glu Ala Gln Leu Thr Pro Arg Val Val Glu Arg Trp Gln Lys Ser
    370                 375                 380

Met Thr Asn Arg Thr Arg Glu Val Leu Leu Pro Lys Phe Lys Leu Glu
385                 390                 395                 400

Lys Asn Tyr Asn Leu Val Glu Ser Leu Lys Leu Met Gly Ile Arg Met
                405                 410                 415

Leu Phe Asp Lys Asn Gly Asn Met Ala Gly Ile Ser Asp Gln Arg Ile
            420                 425                 430

Ala Ile Asp Leu Phe Lys His Gln Gly Thr Ile Thr Val Asn Glu Glu
        435                 440                 445

Gly Thr Gln Ala Thr Thr Val Thr Thr Val Gly Phe Met Pro Leu Ser
    450                 455                 460

Thr Gln Val Arg Phe Thr Val Asp Arg Pro Phe Leu Phe Leu Ile Tyr
465                 470                 475                 480

Glu His Arg Thr Ser Cys Leu Leu Phe Met Gly Arg Val Ala Asn Pro
                485                 490                 495

Ser Arg Ser
```

<210> 57
<211> 232
<212> PRT
<213> Homo sapiens

<400> 57

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                  10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
    130                 135                 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145                 150                 155                 160

Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp
                165                 170                 175

Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys
            180                 185                 190

His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn
        195                 200                 205

Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr
    210                 215                 220

Cys Arg Cys Asp Lys Pro Arg Arg
225                 230
```

<210> 58
<211> 211
<212> PRT

<213> Homo sapiens

<400> 58

```
Lys Asp Gln Gln Arg His Lys Val Arg Glu Glu Val Val Thr Val Gly
1               5                  10                  15

Asn Ser Val Asn Glu Gly Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe
            20                  25                  30

Asp Pro Tyr Thr Val Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg
        35                  40                  45

Met Ser Glu Ser Gly Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly
    50                  55                  60

Ser Gly His Phe Arg Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly
65                  70                  75                  80

Val Asn Tyr Lys Ile Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly
                85                  90                  95

Gln Met Met Ser Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys
            100                 105                 110

Cys Asp Pro His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His
        115                 120                 125

Val Gly Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys
    130                 135                 140

Thr Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg
145                 150                 155                 160

Pro Gly Gly Glu Pro Ser Pro Glu Gly Thr Thr Gly Gln Ser Tyr Asn
                165                 170                 175

Gln Tyr Ser Gln Arg Tyr His Gln Arg Thr Asn Thr Asn Val Asn Cys
            180                 185                 190

Pro Ile Glu Cys Phe Met Pro Leu Asp Val Gln Ala Asp Arg Glu Asp
        195                 200                 205

Ser Arg Glu
    210
```

<210> 59
<211> 2386
<212> PRT

<213> Homo sapiens

<220> misc_feature
<222> (52)..(272)
<223> heparin binding domain

<400> 59

Met Leu Arg Gly Pro Gly Pro Gly Leu Leu Leu Ala Val Gln Cys
1 5 10 15

Leu Gly Thr Ala Val Pro Ser Thr Gly Ala Ser Lys Ser Lys Arg Gln
20 25 30

Ala Gln Gln Met Val Gln Pro Gln Ser Pro Val Ala Val Ser Gln Ser
35 40 45

Lys Pro Gly Cys Tyr Asp Asn Gly Lys His Tyr Gln Ile Asn Gln Gln
50 55 60

Trp Glu Arg Thr Tyr Leu Gly Asn Ala Leu Val Cys Thr Cys Tyr Gly
65 70 75 80

Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro Glu Ala Glu Glu Thr
85 90 95

Cys Phe Asp Lys Tyr Thr Gly Asn Thr Tyr Arg Val Gly Asp Thr Tyr
100 105 110

Glu Arg Pro Lys Asp Ser Met Ile Trp Asp Cys Thr Cys Ile Gly Ala
115 120 125

Gly Arg Gly Arg Ile Ser Cys Thr Ile Ala Asn Arg Cys His Glu Gly
130 135 140

Gly Gln Ser Tyr Lys Ile Gly Asp Thr Trp Arg Arg Pro His Glu Thr
145 150 155 160

Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly Asn Gly Lys Gly Glu
165 170 175

Trp Thr Cys Lys Pro Ile Ala Glu Lys Cys Phe Asp His Ala Ala Gly
180 185 190

Thr Ser Tyr Val Val Gly Glu Thr Trp Glu Lys Pro Tyr Gln Gly Trp
195 200 205

Met Met Val Asp Cys Thr Cys Leu Gly Glu Gly Ser Gly Arg Ile Thr
210 215 220

```
Cys Thr Ser Arg Asn Arg Cys Asn Asp Gln Asp Thr Arg Thr Ser Tyr
225                 230                 235                 240

Arg Ile Gly Asp Thr Trp Ser Lys Lys Asp Asn Arg Gly Asn Leu Leu
                245                 250                 255

Gln Cys Ile Cys Thr Gly Asn Gly Arg Gly Glu Trp Lys Cys Glu Arg
            260                 265                 270

His Thr Ser Val Gln Thr Thr Ser Ser Gly Ser Gly Pro Phe Thr Asp
        275                 280                 285

Val Arg Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro
    290                 295                 300

Tyr Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val Gly Met
305                 310                 315                 320

Gln Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu
                325                 330                 335

Gly Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly
            340                 345                 350

Gly Asn Ser Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly
        355                 360                 365

Arg Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu
    370                 375                 380

Trp Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe
385                 390                 395                 400

Cys Thr Asp His Thr Val Leu Val Gln Thr Gln Gly Gly Asn Ser Asn
                405                 410                 415

Gly Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr
            420                 425                 430

Asp Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr
        435                 440                 445

Thr Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala
    450                 455                 460

Ala His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile
465                 470                 475                 480
```

```
Gly Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys
                485                 490                 495

Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser
            500                 505                 510

Gln Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn
        515                 520                 525

Asp Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr
    530                 535                 540

Cys Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln
545                 550                 555                 560

Cys Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp
                565                 570                 575

Glu Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg
            580                 585                 590

Gly Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser
        595                 600                 605

Ser Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn
    610                 615                 620

Ser His Pro Ile Gln Trp Asn Ala Pro Gln Pro Ser His Ile Ser Lys
625                 630                 635                 640

Tyr Ile Leu Arg Trp Arg Pro Lys Asn Ser Val Gly Arg Trp Lys Glu
                645                 650                 655

Ala Thr Ile Pro Gly His Leu Asn Ser Tyr Thr Ile Lys Gly Leu Lys
            660                 665                 670

Pro Gly Val Val Tyr Glu Gly Gln Leu Ile Ser Ile Gln Gln Tyr Gly
        675                 680                 685

His Gln Glu Val Thr Arg Phe Asp Phe Thr Thr Thr Ser Thr Ser Thr
    690                 695                 700

Pro Val Thr Ser Asn Thr Val Thr Gly Glu Thr Thr Pro Phe Ser Pro
705                 710                 715                 720

Leu Val Ala Thr Ser Glu Ser Val Thr Glu Ile Thr Ala Ser Ser Phe
                725                 730                 735
```

```
Val Val Ser Trp Val Ser Ala Ser Asp Thr Val Ser Gly Phe Arg Val
            740                 745                 750

Glu Tyr Glu Leu Ser Glu Glu Gly Asp Glu Pro Gln Tyr Leu Asp Leu
        755                 760                 765

Pro Ser Thr Ala Thr Ser Val Asn Ile Pro Asp Leu Leu Pro Gly Arg
    770                 775                 780

Lys Tyr Ile Val Asn Val Tyr Gln Ile Ser Glu Asp Gly Glu Gln Ser
785                 790                 795                 800

Leu Ile Leu Ser Thr Ser Gln Thr Thr Ala Pro Asp Ala Pro Pro Asp
                805                 810                 815

Pro Thr Val Asp Gln Val Asp Asp Thr Ser Ile Val Val Arg Trp Ser
            820                 825                 830

Arg Pro Gln Ala Pro Ile Thr Gly Tyr Arg Ile Val Tyr Ser Pro Ser
        835                 840                 845

Val Glu Gly Ser Ser Thr Glu Leu Asn Leu Pro Glu Thr Ala Asn Ser
    850                 855                 860

Val Thr Leu Ser Asp Leu Gln Pro Gly Val Gln Tyr Asn Ile Thr Ile
865                 870                 875                 880

Tyr Ala Val Glu Glu Asn Gln Glu Ser Thr Pro Val Val Ile Gln Gln
                885                 890                 895

Glu Thr Thr Gly Thr Pro Arg Ser Asp Thr Val Pro Ser Pro Arg Asp
            900                 905                 910

Leu Gln Phe Val Glu Val Thr Asp Val Lys Val Thr Ile Met Trp Thr
        915                 920                 925

Pro Pro Glu Ser Ala Val Thr Gly Tyr Arg Val Asp Val Ile Pro Val
    930                 935                 940

Asn Leu Pro Gly Glu His Gly Gln Arg Leu Pro Ile Ser Arg Asn Thr
945                 950                 955                 960

Phe Ala Glu Val Thr Gly Leu Ser Pro Gly Val Thr Tyr Tyr Phe Lys
                965                 970                 975

Val Phe Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln
            980                 985                 990
```

```
Gln Thr Thr Lys Leu Asp Ala Pro  Thr Asn Leu Gln Phe  Val Asn Glu
        995                 1000                1005

Thr Asp  Ser Thr Val Leu Val  Arg Trp Thr Pro Pro  Arg Ala Gln
    1010                1015                1020

Ile Thr  Gly Tyr Arg Leu Thr  Val Gly Leu Thr Arg  Arg Gly Gln
    1025                1030                1035

Pro Arg  Gln Tyr Asn Val Gly  Pro Ser Val Ser Lys  Tyr Pro Leu
    1040                1045                1050

Arg Asn  Leu Gln Pro Ala Ser  Glu Tyr Thr Val Ser  Leu Val Ala
    1055                1060                1065

Ile Lys  Gly Asn Gln Glu Ser  Pro Lys Ala Thr Gly  Val Phe Thr
    1070                1075                1080

Thr Leu  Gln Pro Gly Ser Ser  Ile Pro Pro Tyr Asn  Thr Glu Val
    1085                1090                1095

Thr Glu  Thr Thr Ile Val Ile  Thr Trp Thr Pro Ala  Pro Arg Ile
    1100                1105                1110

Gly Phe  Lys Leu Gly Val Arg  Pro Ser Gln Gly Gly  Glu Ala Pro
    1115                1120                1125

Arg Glu  Val Thr Ser Asp Ser  Gly Ser Ile Val Val  Ser Gly Leu
    1130                1135                1140

Thr Pro  Gly Val Glu Tyr Val  Tyr Thr Ile Gln Val  Leu Arg Asp
    1145                1150                1155

Gly Gln  Glu Arg Asp Ala Pro  Ile Val Asn Lys Val  Val Thr Pro
    1160                1165                1170

Leu Ser  Pro Pro Thr Asn Leu  His Leu Glu Ala Asn  Pro Asp Thr
    1175                1180                1185

Gly Val  Leu Thr Val Ser Trp  Glu Arg Ser Thr Thr  Pro Asp Ile
    1190                1195                1200

Thr Gly  Tyr Arg Ile Thr Thr  Thr Pro Thr Asn Gly  Gln Gln Gly
    1205                1210                1215

Asn Ser  Leu Glu Glu Val Val  His Ala Asp Gln Ser  Ser Cys Thr
    1220                1225                1230
```

```
Phe Asp  Asn Leu Ser Pro Gly  Leu Glu Tyr Asn Val  Ser Val Tyr
    1235                 1240                 1245

Thr Val  Lys Asp Asp Lys Glu  Ser Val Pro Ile Ser  Asp Thr Ile
    1250                 1255                 1260

Ile Pro  Ala Val Pro Pro Pro  Thr Asp Leu Arg Phe  Thr Asn Ile
    1265                 1270                 1275

Gly Pro  Asp Thr Met Arg Val  Thr Trp Ala Pro Pro  Pro Ser Ile
    1280                 1285                 1290

Asp Leu  Thr Asn Phe Leu Val  Arg Tyr Ser Pro Val  Lys Asn Glu
    1295                 1300                 1305

Glu Asp  Val Ala Glu Leu Ser  Ile Ser Pro Ser Asp  Asn Ala Val
    1310                 1315                 1320

Val Leu  Thr Asn Leu Leu Pro  Gly Thr Glu Tyr Val  Val Ser Val
    1325                 1330                 1335

Ser Ser  Val Tyr Glu Gln His  Glu Ser Thr Pro Leu  Arg Gly Arg
    1340                 1345                 1350

Gln Lys  Thr Gly Leu Asp Ser  Pro Thr Gly Ile Asp  Phe Ser Asp
    1355                 1360                 1365

Ile Thr  Ala Asn Ser Phe Thr  Val His Trp Ile Ala  Pro Arg Ala
    1370                 1375                 1380

Thr Ile  Thr Gly Tyr Arg Ile  Arg His His Pro Glu  His Phe Ser
    1385                 1390                 1395

Gly Arg  Pro Arg Glu Asp Arg  Val Pro His Ser Arg  Asn Ser Ile
    1400                 1405                 1410

Thr Leu  Thr Asn Leu Thr Pro  Gly Thr Glu Tyr Val  Val Ser Ile
    1415                 1420                 1425

Val Ala  Leu Asn Gly Arg Glu  Glu Ser Pro Leu Leu  Ile Gly Gln
    1430                 1435                 1440

Gln Ser  Thr Val Ser Asp Val  Pro Arg Asp Leu Glu  Val Val Ala
    1445                 1450                 1455

Ala Thr  Pro Thr Ser Leu Leu  Ile Ser Trp Asp Ala  Pro Ala Val
    1460                 1465                 1470
```

```
Thr Val  Arg Tyr Tyr Arg Ile  Thr Tyr Gly Glu Thr  Gly Gly Asn
    1475                 1480                 1485

Ser Pro  Val Gln Glu Phe Thr  Val Pro Gly Ser Lys  Ser Thr Ala
    1490                 1495                 1500

Thr Ile  Ser Gly Leu Lys Pro  Gly Val Asp Tyr Thr  Ile Thr Val
    1505                 1510                 1515

Tyr Ala  Val Thr Gly Arg Gly  Asp Ser Pro Ala Ser  Ser Lys Pro
    1520                 1525                 1530

Ile Ser  Ile Asn Tyr Arg Thr  Glu Ile Asp Lys Pro  Ser Gln Met
    1535                 1540                 1545

Gln Val  Thr Asp Val Gln Asp  Asn Ser Ile Ser Val  Lys Trp Leu
    1550                 1555                 1560

Pro Ser  Ser Ser Pro Val Thr  Gly Tyr Arg Val Thr  Thr Thr Pro
    1565                 1570                 1575

Lys Asn  Gly Pro Gly Pro Thr  Lys Thr Lys Thr Ala  Gly Pro Asp
    1580                 1585                 1590

Gln Thr  Glu Met Thr Ile Glu  Gly Leu Gln Pro Thr  Val Glu Tyr
    1595                 1600                 1605

Val Val  Ser Val Tyr Ala Gln  Asn Pro Ser Gly Glu  Ser Gln Pro
    1610                 1615                 1620

Leu Val  Gln Thr Ala Val Thr  Asn Ile Asp Arg Pro  Lys Gly Leu
    1625                 1630                 1635

Ala Phe  Thr Asp Val Asp Val  Asp Ser Ile Lys Ile  Ala Trp Glu
    1640                 1645                 1650

Ser Pro  Gln Gly Gln Val Ser  Arg Tyr Arg Val Thr  Tyr Ser Ser
    1655                 1660                 1665

Pro Glu  Asp Gly Ile His Glu  Leu Phe Pro Ala Pro  Asp Gly Glu
    1670                 1675                 1680

Glu Asp  Thr Ala Glu Leu Gln  Gly Leu Arg Pro Gly  Ser Glu Tyr
    1685                 1690                 1695

Thr Val  Ser Val Val Ala Leu  His Asp Asp Met Glu  Ser Gln Pro
    1700                 1705                 1710
```

```
Leu Ile  Gly Thr Gln Ser Thr  Ala Ile Pro Ala Pro  Thr Asp Leu
    1715                 1720                 1725

Lys Phe  Thr Gln Val Thr Pro  Thr Ser Leu Ser Ala  Gln Trp Thr
    1730                 1735                 1740

Pro Pro  Asn Val Gln Leu Thr  Gly Tyr Arg Val Arg  Val Thr Pro
    1745                 1750                 1755

Lys Glu  Lys Thr Gly Pro Met  Lys Glu Ile Asn Leu  Ala Pro Asp
    1760                 1765                 1770

Ser Ser  Ser Val Val Val Ser  Gly Leu Met Val Ala  Thr Lys Tyr
    1775                 1780                 1785

Glu Val  Ser Val Tyr Ala Leu  Lys Asp Thr Leu Thr  Ser Arg Pro
    1790                 1795                 1800

Ala Gln  Gly Val Val Thr Thr  Leu Glu Asn Val Ser  Pro Pro Arg
    1805                 1810                 1815

Arg Ala  Arg Val Thr Asp Ala  Thr Glu Thr Thr Ile  Thr Ile Ser
    1820                 1825                 1830

Trp Arg  Thr Lys Thr Glu Thr  Ile Thr Gly Phe Gln  Val Asp Ala
    1835                 1840                 1845

Val Pro  Ala Asn Gly Gln Thr  Pro Ile Gln Arg Thr  Ile Lys Pro
    1850                 1855                 1860

Asp Val  Arg Ser Tyr Thr Ile  Thr Gly Leu Gln Pro  Gly Thr Asp
    1865                 1870                 1875

Tyr Lys  Ile Tyr Leu Tyr Thr  Leu Asn Asp Asn Ala  Arg Ser Ser
    1880                 1885                 1890

Pro Val  Val Ile Asp Ala Ser  Thr Ala Ile Asp Ala  Pro Ser Asn
    1895                 1900                 1905

Leu Arg  Phe Leu Ala Thr Thr  Pro Asn Ser Leu Leu  Val Ser Trp
    1910                 1915                 1920

Gln Pro  Pro Arg Ala Arg Ile  Thr Gly Tyr Ile Ile  Lys Tyr Glu
    1925                 1930                 1935

Lys Pro  Gly Ser Pro Pro Arg  Glu Val Val Pro Arg  Pro Arg Pro
    1940                 1945                 1950
```

Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu
1955 1960 1965

Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu
1970 1975 1980

Pro Leu Ile Gly Arg Lys Lys Thr Asp Glu Leu Pro Gln Leu Val
1985 1990 1995

Thr Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val
2000 2005 2010

Pro Ser Thr Val Gln Lys Thr Pro Phe Val Thr His Pro Gly Tyr
2015 2020 2025

Asp Thr Gly Asn Gly Ile Gln Leu Pro Gly Thr Ser Gly Gln Gln
2030 2035 2040

Pro Ser Val Gly Gln Gln Met Ile Phe Glu Glu His Gly Phe Arg
2045 2050 2055

Arg Thr Thr Pro Pro Thr Thr Ala Thr Pro Ile Arg His Arg Pro
2060 2065 2070

Arg Pro Tyr Pro Pro Asn Val Gly Glu Glu Ile Gln Ile Gly His
2075 2080 2085

Ile Pro Arg Glu Asp Val Asp Tyr His Leu Tyr Pro His Gly Pro
2090 2095 2100

Gly Leu Asn Pro Asn Ala Ser Thr Gly Gln Glu Ala Leu Ser Gln
2105 2110 2115

Thr Thr Ile Ser Trp Ala Pro Phe Gln Asp Thr Ser Glu Tyr Ile
2120 2125 2130

Ile Ser Cys His Pro Val Gly Thr Asp Glu Glu Pro Leu Gln Phe
2135 2140 2145

Arg Val Pro Gly Thr Ser Thr Ser Ala Thr Leu Thr Gly Leu Thr
2150 2155 2160

Arg Gly Ala Thr Tyr Asn Ile Ile Val Glu Ala Leu Lys Asp Gln
2165 2170 2175

Gln Arg His Lys Val Arg Glu Glu Val Val Thr Val Gly Asn Ser
2180 2185 2190

```
Val Asn  Glu Gly Leu Asn Gln  Pro Thr Asp Asp Ser  Cys Phe Asp
    2195                 2200                 2205

Pro Tyr  Thr Val Ser His Tyr  Ala Val Gly Asp Glu  Trp Glu Arg
    2210                 2215                 2220

Met Ser  Glu Ser Gly Phe Lys  Leu Leu Cys Gln Cys  Leu Gly Phe
    2225                 2230                 2235

Gly Ser  Gly His Phe Arg Cys  Asp Ser Ser Arg Trp  Cys His Asp
    2240                 2245                 2250

Asn Gly  Val Asn Tyr Lys Ile  Gly Glu Lys Trp Asp  Arg Gln Gly
    2255                 2260                 2265

Glu Asn  Gly Gln Met Met Ser  Cys Thr Cys Leu Gly  Asn Gly Lys
    2270                 2275                 2280

Gly Glu  Phe Lys Cys Asp Pro  His Glu Ala Thr Cys  Tyr Asp Asp
    2285                 2290                 2295

Gly Lys  Thr Tyr His Val Gly  Glu Gln Trp Gln Lys  Glu Tyr Leu
    2300                 2305                 2310

Gly Ala  Ile Cys Ser Cys Thr  Cys Phe Gly Gly Gln  Arg Gly Trp
    2315                 2320                 2325

Arg Cys  Asp Asn Cys Arg Arg  Pro Gly Gly Glu Pro  Ser Pro Glu
    2330                 2335                 2340

Gly Thr  Thr Gly Gln Ser Tyr  Asn Gln Tyr Ser Gln  Arg Tyr His
    2345                 2350                 2355

Gln Arg  Thr Asn Thr Asn Val  Asn Cys Pro Ile Glu  Cys Phe Met
    2360                 2365                 2370

Pro Leu  Asp Val Gln Ala Asp  Arg Glu Asp Ser Arg  Glu
    2375                 2380                 2385
```

<210> 60
<211> 662
<212> PRT
<213> Homo sapiens

<400> 60

```
Met Trp Ser Trp Lys Cys Leu Leu Phe Trp Ala Val Leu Val Thr Ala
1               5                  10                  15
```

```
Thr Leu Cys Thr Ala Arg Pro Ser Pro Thr Leu Pro Glu Gln Ala Gln
            20                  25                  30

Pro Trp Gly Ala Pro Val Glu Val Glu Ser Phe Leu Val His Pro Gly
        35                  40                  45

Asp Leu Leu Gln Leu Arg Cys Arg Leu Arg Asp Asp Val Gln Ser Ile
    50                  55                  60

Asn Trp Leu Arg Asp Gly Val Gln Leu Ala Glu Ser Asn Arg Thr Arg
65                  70                  75                  80

Ile Thr Gly Glu Glu Val Glu Val Gln Asp Ser Val Pro Ala Asp Ser
                85                  90                  95

Gly Leu Tyr Ala Cys Val Thr Ser Ser Pro Ser Gly Ser Asp Thr Thr
            100                 105                 110

Tyr Phe Ser Val Asn Val Ser Asp Ala Leu Pro Ser Ser Glu Asp Asp
        115                 120                 125

Asp Asp Asp Asp Asp Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr
    130                 135                 140

Lys Pro Asn Arg Met Pro Val Ala Pro Tyr Trp Thr Ser Pro Glu Lys
145                 150                 155                 160

Met Glu Lys Lys Leu His Ala Val Pro Ala Ala Lys Thr Val Lys Phe
                165                 170                 175

Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr Leu Arg Trp Leu Lys
            180                 185                 190

Asn Gly Lys Glu Phe Lys Pro Asp His Arg Ile Gly Gly Tyr Lys Val
        195                 200                 205

Arg Tyr Ala Thr Trp Ser Ile Ile Met Asp Ser Val Val Pro Ser Asp
    210                 215                 220

Lys Gly Asn Tyr Thr Cys Ile Val Glu Asn Glu Tyr Gly Ser Ile Asn
225                 230                 235                 240

His Thr Tyr Gln Leu Asp Val Val Glu Arg Ser Pro His Arg Pro Ile
                245                 250                 255

Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val Ala Leu Gly Ser Asn
            260                 265                 270
```

```
Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro His Ile Gln
        275                 280                 285

Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys Ile Gly Pro Asp Asn
    290                 295                 300

Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly Val Asn Thr Thr Asp
305                 310                 315                 320

Lys Glu Met Glu Val Leu His Leu Arg Asn Val Ser Phe Glu Asp Ala
                325                 330                 335

Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Leu Ser His His
            340                 345                 350

Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu Glu Arg Pro Ala Val
        355                 360                 365

Met Thr Ser Pro Leu Tyr Leu Glu Ile Ile Ile Tyr Cys Thr Gly Ala
    370                 375                 380

Phe Leu Ile Ser Cys Met Val Gly Ser Val Ile Val Tyr Lys Met Lys
385                 390                 395                 400

Ser Gly Thr Lys Lys Ser Asp Phe His Ser Gln Met Ala Val His Lys
                405                 410                 415

Leu Ala Lys Ser Ile Pro Leu Arg Arg Gln Val Thr Val Ser Ala Asp
            420                 425                 430

Ser Ser Ala Ser Met Asn Ser Gly Val Leu Leu Val Arg Pro Ser Arg
        435                 440                 445

Leu Ser Ser Ser Gly Thr Pro Met Leu Ala Gly Val Ser Glu Tyr Glu
    450                 455                 460

Leu Pro Glu Asp Pro Arg Trp Glu Leu Pro Arg Asp Arg Leu Val Leu
465                 470                 475                 480

Gly Lys Pro Leu Gly Glu Gly Cys Phe Gly Gln Val Val Leu Ala Glu
                485                 490                 495

Ala Ile Gly Leu Asp Lys Asp Lys Pro Asn Arg Val Thr Lys Val Ala
            500                 505                 510

Val Lys Met Leu Lys Ser Asp Ala Thr Glu Lys Asp Leu Ser Asp Leu
        515                 520                 525
```

Ile Ser Glu Met Glu Met Met Lys Met Ile Gly Lys His Lys Asn Ile
530 535 540

Ile Asn Leu Leu Gly Ala Cys Thr Gln Asp Gly Pro Leu Tyr Val Ile
545 550 555 560

Val Glu Tyr Ala Ser Lys Gly Asn Leu Arg Glu Tyr Leu Gln Ala Arg
565 570 575

Arg Pro Pro Gly Leu Glu Tyr Cys Tyr Asn Pro Ser His Asn Pro Glu
580 585 590

Glu Gln Leu Ser Ser Lys Asp Leu Val Ser Cys Ala Tyr Gln Val Ala
595 600 605

Arg Gly Met Glu Tyr Leu Ala Ser Lys Lys Val Trp Asn Leu Lys Ala
610 615 620

Pro Leu Val His Thr Pro Arg Pro Gly Ser Gln Glu Cys Pro Gly Asp
625 630 635 640

Arg Gly Gln Cys Asp Glu Asp Ser Arg Leu Trp Pro Arg Thr Gly His
645 650 655

Ser Pro His Arg Leu Leu
660

<210> 61
<211> 317
<212> PRT
<213> Homo sapiens

<400> 61

Met Lys Val Leu Trp Ala Ala Leu Leu Val Thr Phe Leu Ala Gly Cys
1 5 10 15

Gln Ala Lys Val Glu Gln Ala Val Glu Thr Glu Pro Glu Pro Glu Leu
20 25 30

Arg Gln Gln Thr Glu Trp Gln Ser Gly Gln Arg Trp Glu Leu Ala Leu
35 40 45

Gly Arg Phe Trp Asp Tyr Leu Arg Trp Val Gln Thr Leu Ser Glu Gln
50 55 60

Val Gln Glu Glu Leu Leu Ser Ser Gln Val Thr Gln Glu Leu Arg Ala
65 70 75 80

```
Leu Met Asp Glu Thr Met Lys Glu Leu Lys Ala Tyr Lys Ser Glu Leu
                85                  90                  95

Glu Glu Gln Leu Thr Pro Val Ala Glu Glu Thr Arg Ala Arg Leu Ser
            100                 105                 110

Lys Glu Leu Gln Ala Ala Gln Ala Arg Leu Gly Ala Asp Met Glu Asp
        115                 120                 125

Val Cys Gly Arg Leu Val Gln Tyr Arg Gly Glu Val Gln Ala Met Leu
    130                 135                 140

Gly Gln Ser Thr Glu Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg
145                 150                 155                 160

Lys Leu Arg Lys Arg Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg
                165                 170                 175

Leu Ala Val Tyr Gln Ala Gly Ala Arg Glu Gly Ala Glu Arg Gly Leu
            180                 185                 190

Ser Ala Ile Arg Glu Arg Leu Gly Pro Leu Val Glu Gln Gly Arg Val
        195                 200                 205

Arg Ala Ala Thr Val Gly Ser Leu Ala Gly Gln Pro Leu Gln Glu Arg
    210                 215                 220

Ala Gln Ala Trp Gly Glu Arg Leu Arg Ala Arg Met Glu Glu Met Gly
225                 230                 235                 240

Ser Arg Thr Arg Asp Arg Leu Asp Glu Val Lys Glu Gln Val Ala Glu
                245                 250                 255

Val Arg Ala Lys Leu Glu Glu Gln Ala Gln Gln Ile Arg Leu Gln Ala
            260                 265                 270

Glu Ala Phe Gln Ala Arg Leu Lys Ser Trp Phe Glu Pro Leu Val Glu
        275                 280                 285

Asp Met Gln Arg Gln Trp Ala Gly Leu Val Glu Lys Val Gln Ala Ala
    290                 295                 300

Val Gly Thr Ser Ala Ala Pro Val Pro Ser Asp Asn His
305                 310                 315
```

<210> 62
<211> 8815
<212> DNA

<213> Homo sapiens

<400> 62

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ggggacctgc agccacaact tctctggtcc tctgcatccc ttctgtccct ccacccgtcc 120

ccttccccac cctctggccc ccaccttctt ggaggcgaca acccccggga ggcattagaa 180

gggatttttc ccgcaggttg cgaagggaag caaacttggt ggcaacttgc ctcccggtgc 240

gggcgtctct cccccaccgt ctcaacatgc ttaggggtcc ggggcccggg ctgctgctgc 300

tggccgtcca gtgcctgggg acagcggtgc cctccacggg agcctcgaag agcaagaggc 360

aggctcagca aatggttcag ccccagtccc cggtggctgt cagtcaaagc aagcccggtt 420

gttatgacaa tggaaaacac tatcagataa atcaacagtg ggagcggacc tacctaggca 480

atgcgttggt ttgtacttgt tatggaggaa gccgaggttt taactgcgag agtaaacctg 540

aagctgaaga gacttgcttt gacaagtaca ctgggaacac ttaccgagtg ggtgacactt 600

atgagcgtcc taaagactcc atgatctggg actgtacctg catcggggct gggcgaggga 660

gaataagctg taccatcgca aaccgctgcc atgaaggggg tcagtcctac aagattggtg 720

acacctggag gagaccacat gagactggtg gttacatgtt agagtgtgtg tgtcttggta 780

atggaaaagg agaatggacc tgcaagccca tagctgagaa gtgttttgat catgctgctg 840

ggacttccta tgtggtcgga gaaacgtggg agaagcccta ccaaggctgg atgatggtag 900

attgtacttg cctgggagaa ggcagcggac gcatcacttg cacttctaga aatagatgca 960

acgatcagga cacaaggaca tcctatagaa ttggagacac ctggagcaag aaggataatc 1020

gaggaaacct gctccagtgc atctgcacag gcaacggccg aggagagtgg aagtgtgaga 1080

ggcacacctc tgtgcagacc acatcgagcg gatctggccc cttcaccgat gttcgtgcag 1140

ctgtttacca accgcagcct caccccccagc ctcctcccta tggccactgt gtcacagaca 1200

gtggtgtggt ctactctgtg gggatgcagt ggctgaagac acaaggaaat aagcaaatgc 1260

tttgcacgtg cctgggcaac ggagtcagct gccaagagac agctgtaacc cagacttacg 1320

gtggcaactc aaatggagag ccatgtgtct taccattcac ctacaatggc aggacgttct 1380

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ctgattgcac ttctgagggc agaagagaca acatgaagtg gtgtgggacc acacagaact 1620

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<210> 63
<211> 215
<212> PRT
<213> Homo sapiens

<400> 63

Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Gly Lys Met Ser Ser Tyr
1 5 10 15

Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
20 25 30

Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
35 40 45

Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala

50 55 60

Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
65 70 75 80

Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
85 90 95

Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
100 105 110

Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
115 120 125

Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
130 135 140

Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
145 150 155 160

Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
165 170 175

Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp Glu Glu
180 185 190

Asp Glu Glu Asp Glu Glu Glu Glu Glu Asp Glu Glu Asp Glu Asp Glu
195 200 205

Glu Glu Asp Asp Asp Asp Glu
210 215

<210> 64
<211> 344
<212> PRT
<213> Homo sapiens

<400> 64

Met Val Arg Ala Arg His Gln Pro Gly Gly Leu Cys Leu Leu Leu Leu
1 5 10 15

Leu Leu Cys Gln Phe Met Glu Asp Arg Ser Ala Gln Ala Gly Asn Cys
20 25 30

Trp Leu Arg Gln Ala Lys Asn Gly Arg Cys Gln Val Leu Tyr Lys Thr
35 40 45

Glu Leu Ser Lys Glu Glu Cys Cys Ser Thr Gly Arg Leu Ser Thr Ser

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    50                  55                  60

Trp Thr Glu Glu Asp Val Asn Asp Asn Thr Leu Phe Lys Trp Met Ile
65                  70                  75                  80

Phe Asn Gly Gly Ala Pro Asn Cys Ile Pro Cys Lys Glu Thr Cys Glu
                85                  90                  95

Asn Val Asp Cys Gly Pro Gly Lys Lys Cys Arg Met Asn Lys Lys Asn
            100                 105                 110

Lys Pro Arg Cys Val Cys Ala Pro Asp Cys Ser Asn Ile Thr Trp Lys
        115                 120                 125

Gly Pro Val Cys Gly Leu Asp Gly Lys Thr Tyr Arg Asn Glu Cys Ala
    130                 135                 140

Leu Leu Lys Ala Arg Cys Lys Glu Gln Pro Glu Leu Glu Val Gln Tyr
145                 150                 155                 160

Gln Gly Arg Cys Lys Lys Thr Cys Arg Asp Val Phe Cys Pro Gly Ser
                165                 170                 175

Ser Thr Cys Val Val Asp Gln Thr Asn Asn Ala Tyr Cys Val Thr Cys
            180                 185                 190

Asn Arg Ile Cys Pro Glu Pro Ala Ser Ser Glu Gln Tyr Leu Cys Gly
        195                 200                 205

Asn Asp Gly Val Thr Tyr Ser Ser Ala Cys His Leu Arg Lys Ala Thr
    210                 215                 220

Cys Leu Leu Gly Arg Ser Ile Gly Leu Ala Tyr Glu Gly Lys Cys Ile
225                 230                 235                 240

Lys Ala Lys Ser Cys Glu Asp Ile Gln Cys Thr Gly Gly Lys Lys Cys
                245                 250                 255

Leu Trp Asp Phe Lys Val Gly Arg Gly Arg Cys Ser Leu Cys Asp Glu
            260                 265                 270

Leu Cys Pro Asp Ser Lys Ser Asp Glu Pro Val Cys Ala Ser Asp Asn
        275                 280                 285

Ala Thr Tyr Ala Ser Glu Cys Ala Met Lys Glu Ala Ala Cys Ser Ser
    290                 295                 300
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Gly Val Leu Leu Glu Val Lys His Ser Gly Ser Cys Asn Ser Ile Ser
305 310 315 320

Glu Asp Thr Glu Glu Glu Glu Glu Asp Glu Asp Gln Asp Tyr Ser Phe
325 330 335

Pro Ile Ser Ser Ile Leu Glu Trp
340

<210> 65
<211> 475
<212> PRT
<213> Homo sapiens

<400> 65

Met Glu Ser Lys Ala Leu Leu Val Leu Thr Leu Ala Val Trp Leu Gln
1 5 10 15

Ser Leu Thr Ala Ser Arg Gly Gly Val Ala Ala Ala Asp Gln Arg Arg
20 25 30

Asp Phe Ile Asp Ile Glu Ser Lys Phe Ala Leu Arg Thr Pro Glu Asp
35 40 45

Thr Ala Glu Asp Thr Cys His Leu Ile Pro Gly Val Ala Glu Ser Val
50 55 60

Ala Thr Cys His Phe Asn His Ser Ser Lys Thr Phe Met Val Ile His
65 70 75 80

Gly Trp Thr Val Thr Gly Met Tyr Glu Ser Trp Val Ser Lys Leu Val
85 90 95

Ala Ala Leu Tyr Lys Arg Glu Pro Asp Ser Asn Val Ile Val Val Asp
100 105 110

Trp Leu Ser Arg Ala Gln Glu His Tyr Pro Val Ser Ala Gly Tyr Thr
115 120 125

Lys Leu Val Gly Gln Asp Val Ala Arg Phe Ile Asn Trp Met Glu Glu
130 135 140

Glu Phe Asn Tyr Pro Leu Asp Asn Val His Leu Leu Gly Tyr Ser Leu
145 150 155 160

Gly Ala His Ala Ala Gly Ile Ala Gly Ser Leu Thr Asn Lys Lys Val
165 170 175

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Asn Arg Ile Thr Gly Leu Asp Pro Ala Gly Pro Asn Phe Glu Tyr Ala
            180                 185                 190

Glu Ala Pro Ser Arg Leu Ser Pro Asp Asp Ala Asp Phe Val Asp Val
        195                 200                 205

Leu His Thr Phe Thr Arg Gly Ser Pro Gly Arg Ser Ile Gly Ile Gln
    210                 215                 220

Lys Pro Val Gly His Val Asp Ile Tyr Pro Asn Gly Gly Thr Phe Gln
225                 230                 235                 240

Pro Gly Cys Asn Ile Gly Glu Ala Ile Arg Val Ile Ala Glu Arg Gly
                245                 250                 255

Leu Gly Asp Val Asp Gln Leu Val Lys Cys Ser His Glu Arg Phe Ile
            260                 265                 270

His Leu Phe Ile Asp Ser Leu Leu Asn Glu Glu Asn Pro Ser Lys Ala
        275                 280                 285

Tyr Arg Cys Ser Ser Lys Glu Ala Phe Glu Lys Gly Leu Cys Leu Ser
    290                 295                 300

Cys Arg Lys Asn Arg Cys Asn Asn Leu Gly Tyr Glu Ile Asn Lys Val
305                 310                 315                 320

Arg Ala Lys Arg Ser Ser Lys Met Tyr Leu Lys Thr Arg Ser Gln Met
                325                 330                 335

Pro Tyr Lys Val Phe His Tyr Gln Val Lys Ile His Phe Ser Gly Thr
            340                 345                 350

Glu Ser Glu Thr His Thr Asn Gln Ala Phe Glu Ile Ser Leu Tyr Gly
        355                 360                 365

Thr Val Ala Glu Ser Glu Asn Ile Pro Phe Thr Leu Pro Glu Val Ser
    370                 375                 380

Thr Asn Lys Thr Tyr Ser Phe Leu Ile Tyr Thr Glu Val Asp Ile Gly
385                 390                 395                 400

Glu Leu Leu Met Leu Lys Leu Lys Trp Lys Ser Asp Ser Tyr Phe Ser
                405                 410                 415

Trp Ser Asp Trp Trp Ser Ser Pro Gly Phe Ala Ile Gln Lys Ile Arg
            420                 425                 430
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Val Lys Ala Gly Glu Thr Gln Lys Lys Val Ile Phe Cys Ser Arg Glu
435 440 445

Lys Val Ser His Leu Gln Lys Gly Lys Ala Pro Ala Val Phe Val Lys
450 455 460

Cys His Asp Lys Ser Leu Asn Lys Lys Ser Gly
465 470 475

<210> 66
<211> 226
<212> PRT
<213> Homo sapiens

<400> 66

Met Ser Val Pro Leu Leu Thr Asp Ala Ala Thr Val Ser Gly Ala Glu
1 5 10 15

Arg Glu Thr Ala Ala Val Ile Phe Leu His Gly Leu Gly Asp Thr Gly
20 25 30

His Ser Trp Ala Asp Ala Leu Ser Thr Ile Arg Leu Pro His Val Lys
35 40 45

Tyr Ile Cys Pro His Ala Pro Arg Ile Pro Val Thr Leu Asn Met Lys
50 55 60

Met Val Met Pro Ser Trp Phe Asp Leu Met Gly Leu Ser Pro Asp Ala
65 70 75 80

Pro Glu Asp Glu Ala Gly Ile Lys Lys Ala Ala Glu Asn Ile Lys Ala
85 90 95

Leu Ile Glu His Glu Met Lys Asn Gly Ile Pro Ala Asn Arg Ile Val
100 105 110

Leu Gly Gly Phe Ser Gln Gly Gly Ala Leu Ser Leu Tyr Thr Ala Leu
115 120 125

Thr Cys Pro His Pro Leu Ala Gly Ile Val Ala Leu Ser Cys Trp Leu
130 135 140

Pro Leu His Arg Ala Phe Pro Gln Ala Ala Asn Gly Ser Ala Arg Thr
145 150 155 160

Trp Pro Tyr Ser Ser Ala Met Gly Ser Trp Thr Pro Trp Leu Pro Val
165 170 175

Arg Phe Gly Ala Leu Thr Ala Glu Lys Leu Arg Ser Val Val Thr Pro
180 185 190

Ala Arg Val Gln Phe Lys Thr Tyr Pro Gly Val Met His Ser Ser Cys
195 200 205

Pro Gln Glu Met Ala Ala Val Lys Glu Phe Leu Glu Lys Leu Leu Pro
210 215 220

Pro Val
225

<210> 67
<211> 497
<212> PRT
<213> Homo sapiens

<400> 67

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1 5 10 15

Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
20 25 30

Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
35 40 45

Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
50 55 60

Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
65 70 75 80

Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
85 90 95

Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
100 105 110

Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
115 120 125

Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
130 135 140

Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
145 150 155 160

Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln

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                165                 170                 175

Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
            180                 185                 190

Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
        195                 200                 205

Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
    210                 215                 220

Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
225                 230                 235                 240

Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
                245                 250                 255

Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Val Leu Leu Lys
            260                 265                 270

Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val
        275                 280                 285

Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn
    290                 295                 300

Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly
305                 310                 315                 320

Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe
                325                 330                 335

Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly
            340                 345                 350

Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg
        355                 360                 365

Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala
    370                 375                 380

Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr
385                 390                 395                 400

Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser
                405                 410                 415
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Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp
420 425 430

Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe
435 440 445

Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly
450 455 460

Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly
465 470 475 480

Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp
485 490 495

Phe

<210> 68
<211> 419
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<222> (156)..(156)
<223> Xaa = any or no amino acid

<400> 68

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
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Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe
20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
35 40 45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
50 55 60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65 70 75 80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
85 90 95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
100 105 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys

115 120 125

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
130 135 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Xaa Val Ser Val Tyr
145 150 155 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
165 170 175

Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
180 185 190

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
195 200 205

Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
210 215 220

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225 230 235 240

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
245 250 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
260 265 270

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
275 280 285

Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
290 295 300

Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
305 310 315 320

Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
325 330 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
355 360 365

```
Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
    370                 375                 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385                 390                 395                 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
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Gln Met Ser
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<210> 69
<211> 180
<212> PRT
<213> Homo sapiens

<400> 69

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Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1               5                   10                  15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
            20                  25                  30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
        35                  40                  45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
    50                  55                  60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65                  70                  75                  80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
                85                  90                  95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
            100                 105                 110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
        115                 120                 125

Glu Cys Arg Gly Arg Asn Gly Lys Lys Gln Gln Arg Lys Pro Leu Arg
    130                 135                 140

Glu Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg
145                 150                 155                 160

Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys His Leu Cys Gly Asp Ala
                165                 170                 175
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Val Pro Arg Arg
180

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(71) Applicants *(for all designated States except US)*: **LUDWIG INSTITUTE FOR CANCER RESEARCH** [US/US]; 605 Third Avenue, New York, NY 10158 (US). **LICENTIA, LTD.** [FI/FI]; Erottajankatu 19 B, 6th floor, FIN-00130 Helsinki (FI).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ALITALO, Kari** [FI/FI]; Nyyrikintie 4 A, 02100 Espoo (FI). **HE, Yulong** [CN/FI]; Molecular/Cancer Biology Laboratory,, Biomedicum Helsinki, University of Helsinki, P.O. Box 63 (Haartmaninkatu 8), FIN-00014 Helsinki (FI). **TAMMELA, Toumas** [FI/FI]; Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, University of Helsinki, P.O. Box 63 (Haartmaninkatu 8), Fin-00014 Helsinki (FI).

(74) Agent: **GASS, Daciv, A.**; Marshall, Gerstein & Borun, LLP, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).

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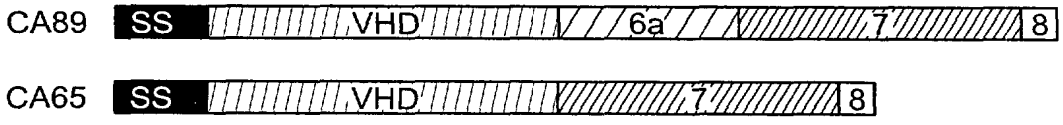
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HEPARIN BINDING VEGER-3 LIGANDS



(57) Abstract: The present invention is directed to methods and compositions for making and using chimeric polypeptides that comprise a VEGFR-3 ligand and a heparin binding domain. The chimeric molecules of the present invention retain VEGFR-3 binding activity and an enhanced heparin binding activity as compared to native VEGF-C and/or VEFG-D.

WO 2005/016963 A3

INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, Sequence Search, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SAARISTO ANNE ET AL: "Lymphangiogenic gene therapy with minimal blood vascular side effects" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 196, no. 6, 16 September 2002 (2002-09-16), pages 719-730, XP002314489 ISSN: 0022-1007 the whole document	1-38

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
21 January 2005	14/02/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Didelon, F

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/019122

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOUKOV V ET AL: "A RECOMBINANT MUTANT VASCULAR ENDOTHELIAL GROWTH FACTOR-C THAT HAS LOST VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 BINDING, ACTIVATION, AND VASCULAR PERMEABILITY ACTIVITIES" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 273, no. 12, 20 March 1998 (1998-03-20), pages 6599-6602, XP002066366 ISSN: 0021-9258 the whole document	1-38
P,A	HE Y ET AL: "Role of lymphangiogenic factors in tumor metastasis" BBA - REVIEWS ON CANCER, ELSEVIER SCIENCE BV, AMSTERDAM, NL, vol. 1654, no. 1, 4 March 2004 (2004-03-04), pages 3-12, XP004492471 ISSN: 0304-419X the whole document	1-38
P,A	JELTSCH MICHAEL ET AL: "Genesis and pathogenesis of lymphatic vessels." CELL & TISSUE RESEARCH, vol. 314, no. 1, October 2003 (2003-10), pages 69-84, XP002314490 ISSN: 0302-766X the whole document	1-38
T	SAHARINEN P ET AL: "Lymphatic vasculature: development, molecular regulation and role in tumor metastasis and inflammation" TRENDS IN IMMUNOLOGY, ELSEVIER, CAMBRIDGE, GB, vol. 25, no. 7, 1 July 2004 (2004-07-01), pages 387-395, XP004518791 ISSN: 1471-4906 the whole document	1-38
A	EL-SHEIKH A ET AL: "A novel vascular endothelial growth factor heparin-binding domain substructure binds to glycosaminoglycans in vivo and localizes to tumor microvascular endothelium" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 62, no. 23, 1 December 2002 (2002-12-01), pages 7118-7123, XP002279013 ISSN: 0008-5472 the whole document	1-38

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/019122

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where ap propriate, of the relevant passages	Relevant to claim No.
A	MULLOY BARBARA ET AL: "Order out of complexity: Protein structures that interact with heparin" CURRENT OPINION IN STRUCTURAL BIOLOGY, vol. 11, no. 5, October 2001 (2001-10), pages 623-628, XP002314491 ISSN: 0959-440X the whole document	1-38